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Weedy adaptation in Setaria spp: genetic diversity, population genetic structure and variation in herbicide resistance

Rong-Lin Wang
Iowa State University

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Wang, Rong-Lin, Ph.D.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

Weedy adaptation in Setaria spp: Genetic diversity, population genetic structure and variation in herbicide resistance

by

Rong-Lin Wang

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For the Graduate College

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To Li and Rachel

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ABSTRACT

Setaria spp. (foxtails) comprise a group of serious cosmopolitan weeds. Current control strategies for these species are flawed due to the heavy reliance on herbicide use and the associated problems. An improved weed management system can only be developed after weedy adaptation is understood, through the studies of weed population biology and physiology. Investigations of Setaria's genetic diversity, population genetic structure, and variation in herbicide resistance represent the initial steps toward this goal.

Isozyme analyses indicated that, foxtails, like other introduced, self-pollinating weeds, had low genetic variation but strong population genetic differentiation. At the species level, S. geniculata (knotroot foxtail, abbreviated herein as Krft) had the greatest heterozygosity, followed by S. viridis (green foxtail, or Gnft), S. glauca (yellow foxtail, or Yeft), and S. faberii (giant foxtail, or Gift). Knotroot foxtail had the strongest population differentiation followed by Yeft and Gnft. Little isozyme polymorphism was found in Gift and no further analysis was conducted for the species. There were geographic patterns in the genetic diversity of individual species. A north-south gradient occurred to Gnft and Krft populations in North America. Yellow foxtail populations formed three distinctive clusters: Asian, European, and North American cluster. Green and Yeft populations in Iowa had greater diversity than those from the rest of North America. Such population genetic structures likely resulted from founder effects, multiple introductions, and natural selection. Some populations, sampled on various geographic scales, were genetically well differentiated. Other populations from diverse ecological environments shared identical genotypes. Phylogenetically, Gnft and foxtail millet were likely the same species and several Gnft varieties appeared to be genetically identical. A high degree of genetic similarity was also shared between Krft and Yeft.

Foxtails had inter- and intraspecific variation in atrazine and metolachlor resistance. The resistance mechanisms (quantitative or qualitative) to these two herbicides may be different in yellow, green and giant foxtail. Glutathione-herbicide conjugation mediated by glutathione S-transferase was not the primary detoxification mechanism for these herbicides in these foxtail species.

No evidence for population shifts to more resistant foxtail variants with prolonged atrazine exposure was found in several detailed studies.

GENERAL INTRODUCTION

Dissertation Organization

This dissertation is organized to have a general introduction, three journal papers (to be submitted), and a general discussion. The general introduction includes an overview, a review of Setaria biology and ecology, a review of weed population biology and a detailed description of the materials and methods. Individual papers have their own brief introductions, materials and methods, results, discussions and references. All other references unrelated to the papers are listed after the general discussion.

Overview

The past several decades have witnessed a dramatic increase in use of agricultural chemicals in the US, from 100,000 metric tons in 1965 to 250,000 metric tons in 1982 (Osteen and Szmedra, 1989). Eighty percent of these chemicals are herbicides, of which, about 90% are used on corn and soybeans. Because of this, weed science has conveniently focused on herbicides, whereas weed biology has largely been ignored. Consequently, weeds including Setaria spp. (foxtails) are poorly understood today in terms of the traits responsible for their success.

These problems have been confounded by the fact that this agronomically important species-group is heterogeneous, containing many species, varieties and forms. The poor understanding of foxtail biology plus the overlooked inherent genetic differences among its members inevitably has led to herbicide applications targeted at the most resistant species or variant in the taxon. This situation has led to heavy reliance on, and excessive use of, herbicides. This overdose situation not only adds more cost to production, but also pollutes the environment. Strong herbicide selection pressure also brings about increasingly resistant biotypes.

To overcome these problems, an alternative strategy in weed management must be developed. This new strategy should be founded upon an understanding of fundamental weed biology and use of integrated approaches to target weak links in the weed's life cycle to undermine weedy competitiveness. This will lead to reduced chemical use with increased

production efficiency. We must study the spatial and temporal distributions of the foxtails, and the traits which underlie their success. Answers to these questions lie at the heart of population biology.

One of the most important aspects in population biology is population genetic structure, which is defined as a nonrandom distribution of genetic variation in natural populations. This structure is the outcome of a complex interaction, over time, between environment (natural selection), and genetics (mutation, random genetic drift and gene flow). Population genetic structure forms the basis of plant spatial and temporal organizations. Successful colonizers may adopt two adaptive strategies in response to environmental changes: locally adapted genotypes ("specialists") through genetic changes, and phenotypic plasticity ("general purpose" genotypes) for adaptive adjustments, particularly in self-pollinating plants (Baker, 1965, 1974; Barrett and Richardson, 1986; Bradshaw, 1965). Population genetic structure may provide important clues about weed adaptive strategies. An inference about weed adaptive strategies will facilitate our understanding of the physiological nature of weedy adaptations.

Knowledge of population genetic structure also has other practical implications (Barrett and Husband, 1990). First, it helps to construct the historical process of migration and colonization, and provides insights into the ecological persistence and evolutionary potential of populations in a new habitat. Second, it is important to genetic resource management, for example, in the areas of crop introduction, germplasm conservation and plant breeding. Third, information on the genetic diversity of a weed in a particular area and its likely source region can be of value in indicating predator-host population relationships for biological control strategies. In addition, it could give us a simplified model system (a group or test-array of the most genetically differentiated populations, and/or the most commonly found genotypes, in different geographic regions) to facilitate the study of variation in important adaptive traits found in the foxtails.

Generally, herbicide resistance is defined as the condition whereby a plant withstands a certain dose of a herbicide by means of several mechanisms, such as altered herbicide action sites and enhanced metabolism. Herbicide resistance is an important adaptive trait for weeds and resistant biotypes can

have a major impact on population genetic structure. ‡iations in herbicide resistance facilitates our understanding of wation, the evolution of herbicide resistant biotypes and the develmore efficient weed management strategies.

Little information exists about herbicide resistance in. Inter- and intraspecific genetic heterogeneity, wide spatial and teribution, and long term exposure to herbicides make it likely that sucoccurs within and among foxtail species. Investigation of its existencmechanisms underlying it) is therefore another focus of this project. id metolachlor were chosen in this study because they re major classes of herbicides recommended for effective contrcduring the last two decades.

The Biology and Ecology of Seta

Foxtails are predominantly self-pollinating, annual, rasses (East, 1940). They depend on their seeds for survival, multipi invasion of new areas. They are characteristic colonizers of disturiems, in particular, cultivated land (Rominger, 1962; Pohl, 1951)hey tend to occur as an associated complex of several species. Thant component of this complex in Iowa agroecosystems is ‡ant foxtail, or Gift). Varying amounts of S. viridis (green foxtail, or Gnauca (yellow foxtail or Yeft) are associated with Gift. Depending on ay of complex and interacting environmental factors, the relative amouspecies in a particular field can vary. Some of these factors are soilage, tillage, moisture, temperature, climatic zone and so on. Both G are competitive in dry and barren soils while Gift favors moisoils (Douglas et al., 1985; Rominger, 1962; Steel et al., 198

Taxonomy and heterogeneity

The grass family (Poaceae) is probably the most imp family to humans. It provides most of our food crops and many fes. A member of Panicoideae, Setaria has approximately 12‡stributed throughout the world (Rominger, 1962). North Americas 43 species and 4 varieties. They are grouped into 3 subgenera: Ptn (6 spp.),

Paurochaetium (10 spp.) and Setaria (27 spp. and 4 varieties). Species in Setaria range from cultivated grains, perennial forage grasses to noxious weeds. Major weedy species include Gnft, Gift, Yeft, S. verticillata and S. geniculata (Krft) (Rominger, 1962).

Taxonomically, foxtails are very complicated. Many taxa and their phylogenetic relationships are still in dispute. Confusion often arises not only at the variety level but also at the species level. It is thus imperative to sort out evolutionary relationships among foxtails as the first step to study foxtail biology. While our focus is primarily on three major weed species, namely Gnft, Gift and Yeft, it will be sometimes necessary to bring other relevant species into the discussion.

It is speculated that Gnft, Gift and S. italica (foxtail millet) are genetically related. Giant foxtail probably originated from hybridization between Gnft and some unknown diploid (Li et al., 1942, 1945), making it an allotetraploid. Foxtail millet is regarded either as an independent species, or a subspecies or variety of Gnft (Ascherson and Graebner, 1899; Beauvois, 1812; Briquet, 1910; De Wet et al., 1979; Linnaeus, 1753; Prasada Rao et al., 1987). Knotroot foxtail is morphologically and genetically very similar to, and often confused with, Yeft (Chikara and Gupta, 1980; Rominger, 1962;). The rhizomes of Krft are the only distinctive specific markers and offer very little practical help for identification because they tend to appear late during development or sometimes are not apparent (personal observations).

A great deal of intraspecific variation is also present in foxtails. Some may be environmental in nature, while other variation is heritable. Foxtails vary in chromosome number and morphological characteristics. The basic chromosome number for the genus Setaria is 9 (Darlington et. al., 1955; Singh et al., 1977). Most of these species are allopolyploids. Accordingly, Gnft from North America, South and East Asia have 2n = 18, Gift from North America and Asia have 2n = 36 (4x) and Yeft from Europe, North America and South Asia have various ploidy levels: 2n = 36 (most common), 2n = 72 (8x) and a series of aneuploids (Fairbrothers, 1959; Li et al., 1942, 1945; Khosla and Sharma, 1973; Steel et al., 1983). It is speculated that hybridization and polyploidy at various levels have played important roles in speciation within this genus. All

three species, as well as foxtail millet, are self-pollinated (Darmency et al., 1987a, b; De Cherisey et al., 1985; Pohl, 1951; Steel et al., 1983)

Morphological polymorphism has been widely reported in all three species. The following traits vary commonly: colors of leaves, panicles, and bristles (setae); panicle shape; number of tillers, seed and panicle size, and so on. Green foxtail is probably the most morphologically variable species (Dore et al., 1980; Douglas et al., 1985; Pohl, 1951; Schreiber and Oliver, 1971; Slife, 1954). While some of the variations seem genetic, others are likely due to phenotypic plasticity. Highly variable characteristics include colors of bristles, spikelets, and leaf blades; width and length of leaf blades; curvature of panicles, and surface markings of the fertile lemmas (Fairbrothers, 1959). Stable morphological traits are: spikelet length, panicle length, panicle width and bristle length. Foxtails were also found to be variable in growth characteristics among populations from different locations (Santelmann and Meade, 1961). It was proposed that microenvironment may have a selective role in producing a particular variant (Schoner et al. 1978).

The taxonomic varieties in Gnft include var. viridis (typical form of the species), var. ambigua (Guss) Coss., var. weinmanni Brand., var. major (Gaudin) Pospichel (giant green foxtail), var. robusta-alba Schreiber (robust white foxtail), and var. robusta-purpurea Schreiber (robust purple foxtail), as determined by morphological markers (Douglas et al., 1985). Giant green foxtail was first reported in the US Corn Belt in Iowa and Illinois (Pohl, 1951; Slife, 1954). It was reported as a variant growing in association with Gift, Gnft and Yeft in corn and soybean fields. Its morphology is similar to Gnft but its robust growth is like that of Gift. Giant green foxtail also has a distinctive number of nodes per plant and number of seeds per panicle (Rominger, 1962). Robust purple and robust white foxtails differ from regular Gnft in the color of their bristles, other morphological characteristics, and physiological responses.

There are also three morphological variants described in Gift (Pohl, 1962). The most prevalent has pubescence on the upper leaf surface only. Two other variants are either pubescent or glabrous on both sides of leaves, but with spikelet characters and chromosome numbers characteristic of Gift. The basis for these morphological variations in Gift and Gnft, whether genetic or

phenotypic, is largely unknown. Additionally, triazine resistant biotypes have been found in all three species (Bandeen et al., 1982; De Prado et al., 1989; Thornhill and Dekker, 1993; Warwick, 1991).

A great amount of variation is maintained in natural populations. Some variation is due to phenotypic plasticity, which itself may be under genetic control (Schlichting, 1986). Genes may flow both within and among populations. This is possible because of seed dispersal and cross pollination. There is very little known about organization of genetic variation in foxtail populations. The basic characteristics of several weedy foxtails are summarized in Table 1.

Origin

Although no explicit phylogenetic analysis of Setaria has been published, it has been speculated that Africa is the center of origin of the genus because 74 out of 125 species are found in that continent (Stapf and Hubbard, 1930). Some species probably later migrated to Europe, Asia and South America, from which a number of modern Setaria species were derived. Before its introduction to other continents, the currently cosmopolitan Gnft probably occupied Eurasia and has been considered as the progenitor species to Gift and foxtail millet (Li et al., 1942, 1945). Yellow foxtail is native to Europe (Fernald, 1950; Rousseau et al., 1969), while Gift reportedly originated from China (Pohl, 1951).

Distribution

Foxtails are found mostly in temperate zones within 45°S to 55°N in latitude (Hafliger and Scholz,1980; Lorenzi and Jeffery, 1987). Both globally and domestically, Gnft and Yeft have wider geographic distributions than Gift. Globally, Gnft and Yeft are found in North America, Central America, part of South America (Argentina, Uruguay and Chile), northern Africa, the Middle East, Europe, east Asia, southern Asia and Australia. Giant foxtail is only found in North America, central Europe, Russia, the Middle East and east Asia.

Green foxtail and Yeft occur throughout the US except for Alaska. Giant foxtail is limited to the east, southeast and part of the north central US. Little detail is known regarding the causes and forces which shaped current foxtail distribution. It is speculated that human and animal migration, trade, wind, river

Table 1. A summary of several weedy Setaria species

Species	Ploidy (n = 9)	Mating system	Native range	Life history
S. viridis (green foxtail)	2n = 18	self - pollinating	Eurasia	annual
S. italica (foxtail millet)	2n = 18	self - pollinating	Eurasia	annual
S. faberii (giant foxtail)	2n = 36	self - pollinating	China	annual
S. glauca (yellow foxtail)	2n = 18, 36, 72, aneuploids	self - pollinating	Europe	annual
S. geniculata (knotroot foxtail)	2n = 36, 72	self - pollinating	Central America, northern South America	perennial
S. verticillata	2n = 18, 36, 54	self - pollinating	Eurasia	annual

flow, industrialization and modern farming all likely promoted dissemination.

Agronomic importance

Foxtails are of considerable agronomic importance. Their associations with agriculture date back thousands of years in ancient civilizations as both crops and weeds (Gao and Chen, 1988). Foxtail millet, a very close relative to Gnft, is still widely cultivated in some countries. Foxtail seeds also serve as an important food source to wildlife (Martin et al., 1961). Green foxtail, Yeft and Gift are listed as major weeds worldwide, the second most important in the United States, and Gift as the most important in Iowa (Holm et al, 1977; Iowa Cooperative Extension Survey, 1987, unpublished; Santelmann et al., 1962; Slife, 1954). They form important components of the annual weed vegetation in the US Corn Belt (Hitchcock, 1971; Lorenzi and Jeffery, 1987; Santelmann et al., 1962; Slife, 1954).

Review of Weed Population Biology

Population genetic structure of introduced weeds and other plant species

Many important agricultural weeds are introduced from other regions. These introduced weeds are often annual, self-pollinating colonizers of disturbed habitats. The nature of introduction often results in only a very small

sample of the original gene pool being transported to a new range. Self-pollination makes it easier for these new immigrants to establish themselves and expand their range. There have been some studies of these aggressive colonizers' population biology in general and population genetic structure in particular in recent years using isozyme markers. Recurrent patterns have emerged from these investigations. Introduced weeds, or colonizers, are often polyploids, self-pollinating, low in genetic variation and have marked population differentiation. There is a strong linkage disequilibrium among loci and only a few multilocus associations present within populations (reviewed in Barrett, 1988; Barrett and Richardson, 1986; Barrett and Shore, 1989; Brown and Marshall, 1981; Rice and Jain, 1985).

In weeds and other plant species, the genetic similarities among plant populations based on isozyme studies reflect their geographical distribution (Barrett and Husband, 1990; Bergmann, 1978; Bretting et al., 1990; Lundkvist and Rudin, 1977; Nevo et al., 1979; Wendel and Parks, 1985; Wendel and Percy, 1990; Yang et al., 1977; Yeh and O'Malley, 1980). Genetic differentiation could be correlated with a number of factors such as latitude, longitude and elevation. Such clustering or clinal patterns of populational variation are believed to depend on the direction or frequency of colonization, natural selection or other evolutionary forces. There are, however, ample examples where population differentiation shows no apparent geographic pattern (Barrett and Husband, 1990; Jain and Rai, 1974; Levin, 1975).

Factors influencing population genetic structure

The spatial and temporal organization of genetic variation in plant populations is controlled and influenced by a complex array of factors. Genetic variation is generated by mutation and organized, maintained, eliminated and dispersed through a complex balance between natural selection, migration (gene flow) and random genetic drift (population size). Certain historical, life history and ecological factors also play prominent roles in shaping the patterns of genetic diversity within and among populations.

Mutation Mutation here is discussed in its broadest sense, which includes base pair changes, insertion, deletion, duplication, chromosome translocation and inversion as well as spontaneous polyploidization. Mutation is obviously

the ultimate source of genetic variation, and it provides the raw materials for evolution. Spontaneous mutation rates, however, are very low. It is typically 10^{-4} to 10^{-6} per gene per generation. For allozymes in Drosophlla, this figure is 1.28×10^{-6} (Hartl, 1989). The cumulative effect of this low mutation rate becomes apparent only after a long period of time. Practically speaking, mutation has little effects on allele frequencies.

Random genetic drift, founder effect and genetic bottlenecks A mating population can be considered to have an infinite pool of gametes. In the process of sexual reproduction, only some of the gametes can unite and give rise to a certain number of individuals which constitute the breeding population for the next generation. There is , therefore, a gamete sampling process in every generation. The small sample of gametes is frequently unrepresentative of the entire pool. That is, the allele frequency in the sample may be different from what it is in the gamete pool. When effective population size (N_e) is too small, this sampling error leads to change in allele frequencies from generation to generation, a phenomenon called random genetic drift. Although the random fluctuation of allele frequencies in any single population is difficult to predict, overall patterns for a large number of populations are clear: eventually, most populations will be fixed for one allele in a given locus. Other populations will show a continuum of intermediate allele frequencies. The higher the initial frequency of an allele, the more likely that the allele will be fixed. The larger the sample, the less allelic frequency will fluctuate. Without gene flow, these populations will show maximum differentiation.

The founder effect is the random genetic drift happened to a newly established population by a few immigrants. It causes a severe loss of genetic variation in newly established populations and most of the genetic diversity present occurs among populations, not within. Furthermore, colonizers are likely to go through cyclic establishment-extinction and genetic bottlenecks will subsequently occur, leading to further erosion of genetic variation (Barrett and Shore, 1989).

Gene flow and selection Gene flow in plants can occur through seed dispersal or pollen dispersal. This gene flow is a homogenizing force preventing populational divergence. Species with restricted gene flow should

exhibit greater genetic differentiation than species with widely dispersed pollen and seeds. For populations at different sites, selection leads to populational divergence if their environments are different, but prevents divergence if their environments are similar. Selection occurs when three conditions are met. First, there is phenotypic variation among individuals for some characters. Second, part of this variation must be genetically based. Third, this variation affects survival and reproduction and, ultimately, fitness (Primack and Kang, 1989). Selection forces could be either environmental (e. g., temperature, moisture, and soil type, etc.) or biotic factors (e. g., interspecific competitors, pests, disease, and human activities, etc.).

Of particular interest is the relationship between herbicide selection and the development and maintenance of population genetic structure. Heavy use of herbicides has become indispensable to modern farming. It has a profound impact on weed populations, the most visible being the drastic change in population size both spatially and temporally. This generates periodic genetic bottlenecks and subsequent genetic drift. Genetic variation within populations will be reduced and populational differentiation will be increased. Populations will be driven to gene fixation and herbicide resistant genotypes will build up. Several studies have documented this process (Andersen, 1987; Gasquez and Compoint, 1981; Solymosi and Lehoczki, 1989; Warwick and Black, 1986; Warwick and Marriage, 1982).

Herbicide resistant biotypes have been reported in over 100 weed species (reviewed in Warwick, 1991). Some cross-resistant biotypes were also reported. The resistance is caused by alterations at the cellular site of herbicide action or by changes of uptake, translocation, or metabolism. Establishment of resistant biotypes is govemed by a number of factors including intensity of herbicide selection, farming practices, size of soil seed bank, genetic variation in herbicide resistance, initial mutation frequencies, mode of inheritance of resistance, gene flow, relative fitness of resistant and susceptible biotypes, and so on (Warwick, 1991). Although very little is known regarding genetic variation in herbicide resistance in natural populations, several studies reported their existence in populations not previously exposed

to herbicides (Price et al., 1983; Putwain et al., 1982; Thai et al., 1985). In general, studies about the evolution of herbicide resistance are limited.

Historical, life history and ecological factors The joint action of mutation, migration, selection and drift determines population genetic structure. These forces, however, operate within the historical and biological context of each species. For introduced, self-pollinating weeds, migration and population establishment have profound consequences for population structure because they often involve only a small number of migrants. Genetic drift leads to a loss of genetic variation. Populations will be highly differentiated with only a limited number of multilocus associations generated within populations, as is the case in barnyard grass (Barrett and Shore, 1989). The overall structure of a colonizer in its new habitat will also be determined by the number of different populations in the source region which supplied migrants, and the likelihood of cross-fertilization among them.

Life history and ecological variables also influence population structure. They include the breeding system, floral morphology, pollination mechanisms, seed dispersal, seed dormancy, phenology, the life cycle, geographical range, and others. Of primary significance is the mating system. It has profound effects on the way genetic variation is organized. On its own, it does not change allele frequencies. In a random mating population, genotype frequencies will conform to Hardy-Weinberg expectations. If inbreeding prevails, there will be an excess of homozygotes and strong linkage disequilibrium. In the extreme case of self fertilization, as is often found in grasses, heterozygotes would rarely be present. Deleterious recessive alleles will be exposed and removed from a population by natural selection.

Self-fertilization might be a mechanism which evolved to ensure plant survival under difficult circumstances. Plants are either predominantly selfers, have mixed mating types or are predominantly outcrossers. The rate of a particular mating type for a given species is variable (Hamrick, 1982). A majority of introduced weeds are either selfers or reproduce asexually (reviewed by Barrett and Richardson, 1986). Uniparental reproduction offers great advantages to colonizers. One such advantage is to facilitate the establishment of a founding population in a new habitat after long distance

dispersal, a challenge facing most migrants. It is a challenge because an initial low plant density plus immobility makes pollination and reproduction very difficult. Self-pollination guarantees the success of reproduction even for a single individual in an alien habitat. A second benefit is that self-pollination will preserve multilocus associations already adapted to local environment and allow them to multiply (Allard, 1965, 1975; Stebbins, 1957). Furthermore, self-pollination imposes a reproductive barrier among differently adapted populations and decreases the probability of undesirable gene flow.

There are multiple consequences of self-fertilization. Autogamous populations would have an excess of homozygotes and few heterozygotes. There is restricted gene flow. Recombination is reduced. There will be fewer genotypes in a population, with plants from the same family lineage (a group of plants sharing the same ancestor) being homogeneous for the same genotype. Genetic differentiation will occur among families. If this happens within a population, there will be intrapopulation genetic subdivision. If it happens among populations, it will promote population differentiation. The combined force of random genetic drift, inbreeding and restricted gene flow will likely produce populations which are highly differentiated with little intrapopulational genetic variation. With the involvement of selection, distinctive ecotypes may be more likely to evolve in a self-fertilizing species because once a favorable genotype, or a non-random association of alleles, is formed, it would be kept intact due to low recombination. Effective recombination occurs only in heterozygotes, not in homozygotes (Allard, 1975).

Use of isozyme molecular markers to study population genetic structure

To study populations, genetic markers are needed. Early taxonomic keys for the identification and separation of Setaria spp. were based primarily on morphological markers, floral as well as vegetative, with emphasis on size. Thus, new variants, such as giant green foxtail or the robust foxtails were identified this way (Rominger, 1962; Schreiber and Oliver, 1971). Later, Williams and Schreiber (1976) combined morphological markers with paper chromatography, wherein alcohol-soluble flavonoid compounds were separated and characterized, to detect genotypic differences among eight

species and varieties of Setaria. With the aid of taxonomic distances and Jaccard's correlation coefficients (Sneath and Sokal, 1973), they proposed that Gnft was the ancestral species, from which two groups evolved. One group is the diploids: foxtail millet, giant green foxtail, robust white foxtail, and robust purple foxtail. The other group is the polyploids: Gift, S. verticillata, and Yeft.

Morphological markers (phenotypes) have been used in systematic studies without reference to their heritability. For most morphological characters, however, determination of genotypes is difficult because of the polygenic inheritance, dominance and recessiveness, epistasis, and environmental influences on morphology and on the production of secondary compounds such as anthocyanins.

Isozymes, on the other hand, provide information fundamentally different from morphological markers (Gottlieb, 1971). Differences in electrophoretic mobilities are usually the result of changes in the amino acid sequences of a protein, which generally reflects a change in its coding gene. In addition, isozyme analysis can be performed on a large number of samples with a relatively small amount of tissue. This makes population genetic studies feasible. Third, isozymes are usually codominantly inherited, free of pleiotropic and epistatic interactions, and genotypes are easily recognizable. Isozymes are also more variable than morphological markers. Lastly, isozyme loci are widely dispersed throughout a genome, thus constituting a representative genomic sample (Weeden and Wendel, 1989). Generally speaking, isozyme markers are clearly superior to morphological ones (Gottlieb, 1971).

It has been well established that changes in electrophoretic mobility of isozymes reflect changes in the encoding structural genes, and usually are not due to polymorphism at the post-transcriptional level (Weeden and Wendel, 1989). Furthermore, for a given enzyme in a diploid, a predictable number of loci will be expressed and their subcellular compartmentations are generally stable and consistent. Enzyme subunit structure is generally conserved. These properties make it possible to interpret zymograms from Setaria without a formal study of isozyme inheritance.

Starch gel electrophoresis was developed by Smithies in 1955. Isozymes were soon discovered by Hunter and Markert in 1957 on starch gels. Isozymes

were used as markers to study animal populations beginning in the mid-1960's and plant populations by the early 1970's. Since then, it has been well established that isozyme markers are important tools for studying plant populational genetic structure. Combined with numerical taxonomic analysis, they also help to identify different populations for further characterization.

In spite of the considerable advantages offered, isozymes do have a few limitations (Hartl, 1988). Of the three classes of genes (roughly speaking, genes encoding ribosomal RNA, mRNA, and tRNA), enzymes are coded by class II genes (genes encoding mRNA) only. Within this class there is a wide range of proteins, but only those enzymes with high cellular concentrations are surveyed because they are easy to assay. Their coding loci are therefore hardly a random sample of all the genes in a plant genome. This may result in an overestimate of total genetic variation. On the other hand, genetic variation could be underestimated too because not all base pair mutations lead to amino acid substitutions, and not all such substitutions alter the charge of a protein. Furthermore, a slight change in protein charge may be undetectable in gel electrophoresis. An ultimate solution, of course, is to sequence DNA or examine RAPD or RFLP markers, techniques which are time-consuming and costly.

Isozymes are still considered excellent genetic markers and are widely employed in plant and weed population biology studies (Barrett and Husband, 1990; Barrett and Richardson,1986; Barrett and Shore, 1989; Barrett,1988; Bergmann, 1978; Brown and Marshall, 1981; Clegg and Allard, 1972; Hamrick and Allard, 1975; Kahler and Price 1986; Lundkvist and Rudin, 1977; Marshall and Allard, 1970; Price and Kahler 1983; Rice and Jain, 1985; Wendel and Parks, 1985; Wendel and Percy, 1990; Yang et al., 1977; Yeh and O'Malley, 1980).

Materials and Methods

Isozyme gel electrophoresis

The isozyme starch gel electrophoresis protocol for this study was adapted from Wendel and Weeden (1989). Tests on foxtail tissues were made to determine a number of factors: optimal tissue age, tissue type, extraction buffer,

tissue:extraction buffer ratio, gel buffers, and staining procedures. After determining the best possible combinations of these factors, 39 enzymes were tested for stainability and resolution. This led to the establishment of a three gel, 13 enzyme system: C gel; for triose phosphate isomerase, aspartate aminotransferase, phosphoglucoisomerase, aldolase and glutamate dehydrogenase; D gel; for malate dehydrogenase, phosphoglucomutase, diaphorase, aconitate hydrotase; CT gel; for adenylate kinase, shikimate dehydrogenase, phosphogluconate dehydrogenase, isocitrate dehydrogenase. The composition of the gel buffers was as follows:

C gel: electrode buffer, 0.192 M boric acid, titrated with lithium hydroxide to pH 8.3. Gel buffer, 0.019 M boric acid, 0.004 M LiOH, 0.047 M Tris and 0.007 M citric acid at pH8.3, total volume 345 ml.

CT gel: 0.040 M citric acid monohydrate, pH adjusted to 6.3 with N-(3-aminopropyl)-morpholine. Gel buffer: one part of electrode buffer mixed with 19 parts of water, total volume 500 ml.

D gel: electrode buffer, 0.065 M L-histidine free base titrated to pH 6.5 with citric acid. Gel buffer, one part of electrode buffer mixed with three parts of water, total volume 500 ml.

A step by step description of the protocol is given below.

a. Sample size

After preliminary tests, the number of plants sampled from each population was set at 10, a number appropriate for a self-pollinating species like the foxtails in which little variation was likely to be found within populations. In this way, it was more time and cost efficient to screen fewer plants per population and more populations.

b. Sample preparation

Tissue extracts were made from seedlings at the 2-3 leaf stage. The extraction buffer was made of 50 mM phosphate at pH 7.5, 5% sucrose (W:V) and 14 mM mercaptoethanol (0.1% V:V). The tissue:extraction buffer ratio was 1:1.25. Tissues were homogenized in Eppendorf tubes placed on ice and centrifuged in a refrigerated microfuge at 4°C. The extracts were then stored in a -80°C freezer or analyzed immediately.

c. Gel preparation

Gels were prepared in a 800W microwave. D and CT gels: 62.5 g starch (Sigma Chemical Co., St. Louis, MO), 20 g sucrose. Add 150 ml gel buffer to make a slurry. Shake 5 min on a platform shaker at 140 rpm. Heat the remaining 350 ml of buffer in a microwave for 2.5 min. Add the hot buffer to the starch slurry while it is being vigorously shaken. Heat the preparation for another 3.0 min. De-gas and shake. Pour starch slurry into gel mold. After it cools, wrap the gel with SaranWrap and leave it at room temperature overnight. C gels, 43.1g starch added with 100ml gel buffer to make a slurry. Shake for 4 min. The remaining 245 ml of buffer was heated for 2.5 min. Mix hot buffer to make a starch slurry. Heat for another 1.25 min. De-gas and pour into gel mold.

d. Electrophoresis

Tissue samples were absorbed onto wicks which were cut from Whatman filter paper. Wicks were then applied to the precooled gel (at 4°C for about 20 min.). An electrical potential was then applied across the gel by a power supply (EC400, Constant Power). The D gel ran for 6.5 hr at 16W constant power, C gel ran for 5 hr at 13W, and CT gel ran for 6.0 hr at 16W. Gels were placed in a refrigerated chamber (4°C) and covered by precooled water bags (at 4°C for about 20 min.) during electrophoresis.

e. Enzyme visualization

Following electrophoresis, isozymes were detected in situ with specific enzyme activity stains (Wendel and Weeden, 1989). Gel slices were immersed in staining solutions whereby the substrates and other reagents diffused into the gel where they were catalyzed by the enzyme under study. Detection was based on precipitation of soluble indicator dyes, which became insoluble and colored in zones containing enzyme activity (banding pattern). Tetrazolium salts, diazonium salts or various redox dyes served as indicators. When isozymes were sufficiently stained, the staining solution was poured out and the gel rinsed with water. To avoid loss of clarity in the banding patterns, gels were photographed within minutes of stopping the staining reactions.

Isozyme gel interpretation

The enzyme pattern (number, spacing and intensity) was characteristic of the particular enzyme system assayed and its mode of inheritance. For some

enzymes (usually esterases, phosphatases and peroxidases), individuals may display complex patterns with as many as 10 to 15 bands because multiple isozymes were encoded by numerous genetic loci. In contrast, other enzymes were specified by a single structural gene and individuals may display only a single band.

The number of polypeptide subunits for each enzyme and the allelic state of the coding gene (homozygous or heterozygous) also determined the number of enzyme bands displayed. For an enzyme composed of a single polypeptide (monomeric), an individual heterozygous at the coding gene displayed two allozyme bands, but if the enzyme was dimeric (composed of two polypeptides), three allozymes were displayed, if it were tetrameric, five were displayed, etc.

The number of visible enzyme bands can be reduced if enzymes specified by different genes overlapped on the gel because they had similar mobilities, or if an individual was homozygous for a "null" gene. Artifacts that might result from procedures of extraction or electrophoresis can also change the band number. Ploidy level influenced the number of isozyme bands because polyploids had more genes than diploids and they often coded for electrophoretically distinct enzymes. Foxtail species occur generally at two ploidy levels. Green foxtail is a diploid, and Yeft and Gift tetraploids. Since there is no evidence for gene silencing, tetraploids should be considered to have twice as many loci as a diploid. However, when a gel was scored, the number of loci for tetraploids was determined conservatively based on the apparent banding patterns. Yellow foxtail and Krft were considered as conspecific in this study because of the similarities shared at various isozyme loci and in morphology. The genetic distance between the two species was determined by alleles unique to each one and the difference in allele frequencies.

Based on the genetic inheritance studies of isozymes from other species (reviewed in Weeden and Wendel, 1989), foxtail isozymes were scored as follows:

AAT (Aspartate aminotransferase E. C. 2.6.1.1), dimeric.

S. viridis There are three loci with one allele each. Homozygous in all three loci. Locus one has two alleles. Genotypes: AAT1-AA, AAT1-BB, AAT2-AA, AAT2-BB, and AAT3-AA.

S. glauca There are four detectable loci. Genotypes: AAT1-AA, AAT2-AA, AAT3-AA, AAT3-BB, and AAT4-AA .

S. geniculata There are four detectable loci. Genotypes: AAT1-AA, AAT2-AA, AAT2-BB, AAT3-AA, and AAT4-AA.

ACO (Aconitate hydrotase, E. C. 4.2.1.3), monomeric.

S. viridis There are two loci. The most anodal one is cytosolic. The cathodal one is mitochondrial. Locus one has one allele (top band). The second band is a shadow band (Wendel et al., 1988). Locus two has two alleles. Genotypes: ACO1-AA, ACO1-BB (rare), ACO2-AA, and ACO2-BB.

S. glauca There are four loci. Genotypes: ACO1-AA, ACO2-AA, ACO2-BB, ACO3-AA, ACO3-BB, ACO4-AA, ACO4-BB, and ACO4-CC.

S. geniculata There are four loci. Genotypes: ACO1-AA, ACO2-AA, ACO3-AA, ACO3-BB, ACO3-CC, ACO4-AA, ACO4-BB, and ACO4-CC.

ADK (Adenylate Kinase E. C. 2.7.4.3), monomeric.

S. viridis There is one locus with one allele, located in plastid. Genotype: ADK1-AA.

S. glauca There are two loci. Genotypes: ADK1-AA, ADK1-BB, ADK2-AA, and ADK2-BB.

S. geniculata There are two loci. Genotypes: ADK1-AA, ADK2-AA.

ALD (Aldolase E. C. 4.1.2.13), tetrameric.

S. viridis There is one locus. Genotype: ALD1-AA.

S. giauca There is one locus. Genotype: ALD1-AA.

S. geniculata The same as above.

DIA (Diaphorase E. C. 1.6.99), monomeric.

S. viridis There are two loci. Genotypes: DIA1-AA, DIA1-BB (rare), DIA2-AA, and DIA2-BB (rare).

S. glauca There are 2 loci. Genotypes: DIA1-AA, DIA2-AA, and DIA2-BB.

S. geniculata There are only two detectable loci. Genotypes: DIA1-AA, DIA2-AA, and DIA2-BB.

GDH (Glutamate dehydrogenase E. C. 1.4.1.2), hexameric.

S. viridis There is one locus. Genotype: GDH-1AA.

S. glauca There is one locus. Genotype: GDH-1AA.

S. geniculata the same as above.

IDH (Isocitrate dehydrogenase E. C. 1.1.1.41, 1.1.1.42), dimeric.

S. viridis There are four loci. Genotypes: IDH1-AA, IDH2-AA, IDH3-AA, IDH3-BB, IDH3-CC, and IDH4-AA.

S. glauca There are 4 loci. Genotypes: IDH1-AA, IDH2-AA, IDH3-AA, IDH3-BB, IDH3-CC, and IDH4-AA.

S. geniculata There are four loci. Genotypes: IDH1-AA, IDH2-AA, IDH3-BB, and IDH4-AA.

MDH (Malate dehydrogenase E. C. 1.1.1.37), dimeric.

S. viridis There are four loci in two separate compartments. Genotypes: MDH1-AA, MDH1-BB (rare), MDH2-AA, MDH2-BB (rare), MDH3-AA, MDH4-AA, MDH4-BB, and MDH4-CC.

S. glauca Assuming 4 loci present. Genotypes: MDH1-AA, MDH2-AA, MDH3-AA, MDH4-AA, and MDH4-BB.

S. geniculata Assuming 4 loci. Genotypes: MDH1-AA, MDH1-BB, MDH2-AA, MDH2-BB, MDH3-AA, and MDH4-AA.

PGD (Phosphogluconate dehydrogenase E. C. 1. 1. 1. 4. 4), dimeric.

S. viridis There are three loci. Two enzymes are in the plastid (top bands) and one in the cytosol. Genotypes: PGD1-AA, PGD1-BB, PGD1-CC, PGD2-AA, PGD3-AA, and PGD3-BB (rare). PGD1-BB and PGD2-AA co-migrate to give a one-banded pattern.

S. glauca There are four loci. Genotypes: PGD1-AA, PGD2-AA, PGD3-AA, PGD4-AA, and PGD4-BB (rare).

S. geniculata There are four loci. Genotypes: PGD1-AA, PGD2-AA, PGD3-AA, and PGD4-CC.

PGI (Phosphoglucoisomerase E. C. 5. 3. 1. 9), dimeric.

S. viridis There are three loci. The faster bands (PGI1 and PGI2) are in the plastid and the slower one in the cytosol. Genotypes: PGI1-AA, PGI1-BB, PGI2-AA, PGI3-AA, and PGI3-BB.

S. glauca There are three loci. Genotypes: PGI1-AA, PGI2-AA, and PGI3-AA.

S. geniculata There are three loci. Genotypes: PGI1-AA, PGI2-AA, and PGI3-BB.

PGM (Phosphoglucomutase E. C. 5.4.2.2), monomeric.

S. viridis There is one locus. Genotypes: PGM1-AA, PGM1-BB (rare), and PGM1-CC (rare).

S. glauca There are two loci. The most anodal dark band is PGM1-AA or PGM1-BB. The bottom two bands are PGM2-AA or PGM2-BB.

S. geniculata There are two loci. The most anodal one is PGM1-BB or PGM1-AB. The bottom band is PGM2-BB.

SKD (Shikimate dehydrogenase E. C. 1.1.1.25), monomeric.

S. viridis There is one locus. and located in plastid. SKD1 has two alleles. Each allele has two bands. Genotype SKD1-AA, SKD1-BB, and SKD1-CC.

S. glauca There are two loci. Genotypes: SKD1-AA, SKD1-BB (rare), SKD1-CC (rare), SKD2-AA, SKD2-BB (rare), and SKD2-CC (rare).

S. geniculata There are two loci. Genotypes: SKD1-AA, SKD1-BB, SKD1-CC, SKD2-AA, SKD2-BB, and SKD2-CC.

TPI (Triose phosphate isomerase, E. C. 5. 3. 1. 1), dimeric.

S. viridis There are two loci. The more anodal (faster) one (TPI1) is located in the plastid and the slower one (TPI2) in cytosol. Genotypes: TPI1-AA, TPI1-BB (rare), TPI2-AA, TPI2-BB (rare), and TPI2-CC (rare).

S. glauca There are four loci. Enzyme dimeric. Genotypes: TPI1-AA, TPI2-AA, TPI2-BB (rare), TPI3-AA, TPI3-BB (rare), and TPI4-AA.

S. geniculata There are four loci. Enzyme dimeric. Genotypes: TPI1-AA, TPI2-AA, TPI3-AA, TPI3-BB, and TPI4-AA.

Isozyme data analysis

Enzyme electrophoretic patterns have a well-founded genetic basis as shown by previous studies. Thus variations observed in zymograms reflect genetic variation in the sampled populations. Quantification of genetic variation makes it possible to compare plants interspecifically or intraspecifically. After being scored, a zymogram was converted into genotype data which was then analyzed by two computer software programs, BIOSIS-1 (Swofford and Selander, 1981) and NTSYS (Rohlf, 1991). BIOSYS-1 generated allele frequencies at various loci for each population. Allele frequencies of

polymorphic loci were then used by NTSYS for principal component analysis. Graphical representation of the principal component analysis gave a visual display of the relationships among populations and species. When combined with ecological and geographical factors, these analyses helped to identify putative foxtail variants which then could be subjected to physiological characterization.

Listed below are a number of important parameters calculated in these analyses to describe genetic diversity and population genetic structure (Barrett and Shore, 1989).

a. Allele frequency at each locus by geographic region and species

b. Number of unique alleles

c. Average number of alleles per locus (Brown and Weir, 1983)

This measure emphasizes one component of diversity: allelic richness. It is sensitive to sample size, which makes comparisons among samples difficult.

d. Percentage of polymorphic loci (Brown and Weir, 1983)

This yields a rough estimate of the level of genetic variation in a sample and is dependent on sample size, number and kinds of enzymes surveyed. It is most useful when a large number of loci and a large number of individuals per locus are studied.

e. Mean panmictic heterozygosity (gene diversity; Brown and Weir, 1983)

This parameter emphasizes another component of genetic diversity: allelic evenness. It equals the expected proportion of heterozygous loci in a randomly chosen individual. This is one of the most common measures of diversity. It is the sum of squares of allele frequencies at a locus, subtracted from one, and then averaged over all the loci sampled. It is relatively insensitive to sample size but largely dependent on the frequencies of the two most common alleles. When allele frequencies approach equality, it becomes less sensitive to changes in frequency (Brown and Weir, 1983).

f. Genetic identity and genetic distance

Genetic identity ($\underline{I}$) is a commonly used measure of the mean similarity of all pairs of populations within each species. It gives a good assessment of the degree of population differentiation. Its values vary from 0 to 1. When $\underline{I}$

= 1, all allelic frequencies in the two populations are equal, i. e., genetic divergence is minimum; and when I = 0, the two populations have no alleles in common, i. e., genetic divergence is maximum.

g. Coefficient of genetic differentiation (Nei, 1987)

$G_{ST} = D_{ST}:H_T$

D_{ST}: average gene diversity among subpopulations

H_T: average gene diversity in the total population

G_{ST} indicates the proportion of total genetic variation resulting from differences among populations. It is highly dependent on the value of H_T. When this is small, G_{ST} may be large even if the absolute gene differentiation is small.

Principal component analysis Character states of operational taxonomic units (OTUs) are recorded as a data matrix. In this study, character states are different allele frequencies. This matrix's rows are accessions and the columns are the various alleles. This data matrix makes it possible to compare similarities of different OTUs. Before the analysis proceeds, however, the data matrix must be transformed into a new set of non-correlated, composite characters. A variance-covariance matrix is then derived and the first few principal components are extracted. These components account for most of the variation in original data matrix and so can replace the original. As a result, considerable data simplification can be achieved. This allows researchers to analyze genetic responses involving multiple loci to evolutionary and environmental forces (Wendel and Parks, 1985). The OTUs are scatter-plotted based on the principal component scores, enabling a direct visual examination of the relationship of all OTUs and their possible correlation with geographic parameters (Kent and Coker, 1992).

Hypotheses and goals

I hypothesized that foxtail species, like other predominantly self-pollinating weed species, have low genetic diversity with marked population differentiation. Geographic patterns will be evident in their genetic diversity. Variation in herbicide resistance exists both within and among species. To test these hypotheses, isozyme markers will assess the genetic variation and population genetic structure of foxtails. The most genetically divergent

populations will be characterized for their variability in herbicide resistance by a whole plant dose response assay, an in vivo fluorescence emission assay, and an in vitro glutathione S-transferase assay.

WEEDY ADAPTATION IN SETARIA SPP.: I. ISOZYME ANALYSIS OF THE GENETIC DIVERSITY AND POPULATION GENETIC STRUCTURE IN SETARIA VIRIDIS

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Rong Lin Wang, Jonathan Wendel and Jack Dekker

Abstract: Setaria viridis is an important cosmopolitan weed of temperate regions worldwide. Allozyme markers were used to investigate genetic diversity and structure in 168 accessions (including four S. italica) collected mainly from North America and Eurasia. Genetic diversity in green foxtail, and its population genetic structure, provided important clues about this weed's evolutionary history. Genetic diversity was low, with marked population differentiation: the percentage of polymorphic loci was 25% (0.95 criterion); the mean number of alleles per locus was 1.86 (no criterion); the mean panmictic heterozygosity was 0.07, and coefficient of population genetic differentiation was 0.65. A common genotype occurred in 25 accessions distributed in six countries from both the Old World and New World, in a wide variety of ecological situations. Relatively little genetic divergence occurred between Eurasia and North America, with Nei's unbiased genetic identity between the two regions equalling 1.0. These two continents also had equivalent genetic diversity. Within North America, regional differentiation into northern and southern groups separated at 43.5°N latitude was revealed. No geographic pattern in genetic diversity was found within Eurasia. At the regional level, genetic diversity varied independent of the size of the geographic range. These results suggest that green foxtail population genetic structure in North America is the consequence of multiple introductions followed by local adaptation and regional differentiation due to natural selection in its new range. Finally, S. italica and several green foxtail varieties did not differ isozymatically. This supports the view that S. italica and S. viridis are conspecific, and also questions the taxonomic validity of formally recognizing morphological varieties within green foxtail.

Introduction

Setaria is a grass genus of about 125 species that includes food crops and a number of important weeds. Some of the weedy Setaria species are S. viridis (Linnaeus) Beauvois (green foxtail), S. glauca (Weigel) F. T. Hubbard (yellow foxtail), S. faberii Herrmann (giant foxtail), S. verticillata (Linnaeus) Beauvois, and S. geniculata (Lamarck) Beauvois (knotroot foxtail; Rominger, 1962). Setaria italica (Linnaeus) Beauvois (foxtail millet), a close relative of S. viridis, is an important world grain crop. It has been argued that many temperate weedy foxtails evolved from green foxtail (Li, et al., 1942,1945; Prasado Rao et al., 1987; Rominger, 1962; Werth, 1937; Williams and Schreiber, 1976; Willweber-Kishimoto, 1962). Green foxtail is morphologically heterogeneous, with many variants. The systematic interrelationships between these variants are not clear.

Although there have been no explicit phylogenetic analyses of Setaria, it has been speculated that Africa is the original home of the genus because 74 out of 125 species occur on that continent (Stapf and Hubbard, 1930). Before being introduced to other continents, green foxtail's natural range was probably Eurasia (Li et al, 1942, 1945).

Today green foxtail is primarily a temperate species and is generally found within 45°S to 55°N latitudes (Holm et al., 1977). It is one of the most widely distributed weedy foxtail species both globally and in the United States. Globally, it ranges from North America, through Central America to parts of South America; from Europe to northern Africa, and from east Asia to south Asia and Australia (Hafliger and Scholz, 1980). It is found in every state in the continental U.S., and province in Canada (Lorenzi and Jeffery, 1987).

Foxtails are of considerable agronomic importance. Their associations with agriculture, as both crops and weeds, date back thousands of years to ancient civilizations (Gao and Chen, 1988). Foxtail millet (S. italica) is one of the oldest cultivated cereals of China, dating back about 6000 years to the earliest agricultural settlements of the Yang-shao culture phase (Cheng, 1973). Today, this crop is widely grown in Africa, China, India and scattered areas throughout Eurasia (Gao and Chen, 1988; Kawase and Sakamoto, 1984). Setaria viridis, S. glauca and S. faberii are listed as major weeds worldwide and comprise the second most important weed group in the United States (Holm et al, 1977).

Foxtail seeds also serve as an important food source for wildlife (Martin, et al., 1961). Since their introduction to North America, foxtails have expanded in terms of range, population density and the appearance of new morphological variants. Crop yield losses and herbicide expenditures make control of the foxtails a significant problem in crop production.

Substantial biological diversity, wide geographic distribution, and strong competitiveness in disturbed habitats all mark foxtails as highly successful weeds. There is, however, little understanding of what traits lead to their success as weeds, or the implications and significance of genetic heterogeneity to their adaptation. Answers to these questions require knowledge of both population biology and physiology.

One of the important aspects of population biology is population genetic structure, which forms the basis of plant spatial and temporal organization. Knowledge of population genetic structure has practical implications (Barrett and Husband, 1990). It can be used to reconstruct the historical process of migration and colonization, and provide insights into the ecological persistence and evolutionary potential of populations in new habitats, leading to a better understanding of weedy adaptation. It is also important for genetic resource management, such as in germplasm conservation and plant breeding. Information about the genetic diversity of a species in a particular region can be of value in devising effective biological controls, which match locally-adapted weed genotype-predator pairs. In addition, knowledge of population genetic structure could provide clues to the development of a simplified model system to study adaptive functional traits and overcome the limitations inherent in inter- and intraspecific Setaria spp. heterogeneity.

There have been no comprehensive studies of foxtail population genetic structure using molecular markers. What information is available indicates overall low genetic variation in S. faberii, S. italica and green foxtail (Jusuf and Pernes, 1985; Nguyen and Pernes, 1985; Warwick et al., 1987; Warwick, 1990). Some studies on other introduced weeds indicate that they are low in genetic variation within populations and high in genetic differentiation among populations (Barrett, 1988; Barrett and Richardson, 1986; Barrett and Shore, 1989; Brown and Marshall, 1981; Rice and Jain, 1985). These other species

also exhibit a strong linkage disequilibrium among loci, resulting in a limited number of predominant multilocus associations within populations.

Knowledge of why foxtails are successful weeds is essential for developing future weed management strategies. Current weed management systems rely heavily on herbicides. Problems associated with these chemicals include high annual costs; overdose application as a result of ignorance of weed heterogeneity; selection for herbicide-resistant biotypes and environmental pollution. Development of new approaches require a knowledge of weed population biology. When we understand why weeds are so successful, we can target the weak or important links in the weed life cycle and manage its weedy competitiveness.

Our objectives for this study are: 1) to assess the genetic diversity of green foxtails as a species; 2) to investigate the geographic patterns of genetic diversity at the levels of continents, states, local regions and individual farms; and 3) to understand the systematic relationship between S. italica and various S. viridis varieties. These findings will serve as a basis for discussing weedy adaptation in green foxtail, and the historical developments that may have led to their current population genetic structure.

Materials and Methods

Seed collections

A total of 168 accessions of S. viridis (including a few S. italica) were acquired for this project either through our own plant explorations or from colleagues (Table 1). They came from four continents (North America, Europe, Asia and Africa) representing 13 countries. Most of the foreign accessions were collected in 1992, whereas other accessions were provided to us by the USDA-ARS North Central Regional Plant Introduction Station. Domestically, they originated from 19 states, mostly from east of the Rocky Mountains and west of the Appalachians. They came from as far north as Manitoba and as far south as Oklahoma and Arkansas, the principal agricultural area of the continent. In this region, Iowa and North Dakota received the most extensive sampling. Iowa collections included those from waste areas, roadsides and several farms. These farms (except the Luther farm) all had a history of ca. 20 years of

Table 1. List of 168 S. viridis accessions (including four S. italica); latitude and longitude are the ranges or medians for the nation, province or state; Setaria sp. variety abbreviations: GG-giant green (S. virldis, var. major), RP-robust purple (S. viridis, var. robusta-purpurea), RW-robust white (S. viridis, var. robusta-alba), TR-triazine resistant.

Country and Region		Location	Others	Accession
AFRICA				
South Africa (22-35°S/17-33°E)		unknown	S. italica	1872
ASIA				
Afghanistan (30-39°N/61-71°E)		unknown		1852
China (19-53°N/74-134°E)		unknown		1859
India (9-36°N/70-96°E)		unknown	S. italica	1874
Iran (25-40°N/45-62°E)		unknown		1850, 1851
Japan (31-45°N/130-145°E)	Yamaguchi	Yamaguchi	2405 =S. italica	2405, 2409, 2416
		Hagi	2421 = var. Pachystachys	2419, 2421
	Osaki	Takatsuki City		2433
	Ohita	Kyushu Island		2395
		Nagoya		2392
	Nagano	Ina City		2441
	Yokohama	Yokohama Harbor		2442
Russia (50-78°N/25-180°E)	Chita	Silka		2463
		Cernysevsk		2458
Turkey (36-41°N/26-44°E)		unknown		1853-57
EUROPE				
Belgium (51°N/5°E)		unknown		1858
Czech (47-51°N/12-22°E)	Lednice	Buclarchodnik		2491
	northern Bohemia	Lovosice		2525
	Decin	Novel Loubi Port		2551
		Prague		2500, 2512
		Usti nad Labem		2526,2532, 2542
France (42-51°N/5°W-7°E)		unknown		3017
Germany (47-55°N/6-15°E)		Munich		2558
		Regensburg		2560
Italy (37-47°N/7-18°E)		Verona		2572, 2584
The Netherlands (52°N/6°E)		Wageningen		2591
Russia (50-78°N/25-180°E)		Moscow	2476 = Var. Pycnocoma	2464, 2476
		unknown	S. italica	1873
NORTH AMERICA				
Canada	Manitoba (55°N/98°W)	Darlingford		1471
		Deloraine		1474
		Dunrea		1473
		Gilbert Plains		1477
		Killarney		1472
		Lyleton		1475
		Pierson		1476
		Portage la Prairie		1478
		Reston		1469-70
	Ontario(52°N/88°W)	Elora	26 = T R	1484
		Northumberland		26
		SW Ontario		27
United States	AR (35°N/92°W)	Fayetteville		1749
	CA (36°N/120°W)	Walnut Creek		1625-27

Table 1. (continued)

Country and Region	Location	Others	Accession
CO (37°N/106°W)	Fort Collins		1693
	unknown		1485
IA (42°N/94°W)	Allison		1219
	Ames	1737=GG	110,738,1737
	Blackhawk Co.		1216
	Boone Co.		1271
	Dallas Center		1026,1032
	Decorah		654,893,895-898
	Delaware		1048
	Dubuque		652
	Hampton		658,843,1322
	HolyCross		655-657,660
	unknown		1552
	Keokuk Co.		1144
	Lansing		887, 890
	Luther		1262-64, 1266-68
	Malcom		1038, 1041
	Marble Rock		902
	Moscow		1050
	Otley		1309
	State Center		1739
	Washington		729,730,732
ID (45°N/115°W)	Aberdeen		1623
	American Falls		1624
IN (40°N/86°W)	West Lafayette	GG, RP, RW*	1603-05
KS (38°N/98°W)	Ellis Co.		1694
	unknown		1554
MD (38°N/76°W)	Beltsville		1273
MI (42°N/85°W)	Kalamazoo		1742-43
MN (46°N/92°W)	unknown		1547, 1556
	Rosemont		1622
	South Lake Park		1769
	Waseca		1599
NE (41°N/100°W)	North Platte		1752
	unknown		1553
ND (47°N/100°W)	Colfax		1776 1796-98
	Embden		1781,1799-1802
	Erie		1765-68
	Fargo		1608-12, 1764, 1791-93
	Hillsboro		1806-09,1812
	unknown		1770
	Whapeton		1803-05,1810-11, 1794-95
	Wyndmere		1763
OK (36°N/97°W)	Stillwater		1618
OR (43°N/121°W)	unknown		1705
	Ontario		1813
PA (41°N/77°W)	Penn State Univ.		1628
SD (44°N/100°W)	unknown		1555
WA (47°N/120°W)	Prosser		1596,1598,1697-98,1706
WI (45°N/89°W)	Madison	1278 = GG	1278-79
WY (42°N/107°W)	Laramie		1700

continuous atrazine use. Bulk seeds were collected from several 30 m by 1 m transects beginning from the fence row to 80 m inside the field. The distance between transects varied from about 1 m to 20 m. Seeds from the Luther farm were randomly picked. North Dakota accessions were systematically collected in agricultural fields adjacent to farm roads and highways about every 3 miles, along east-west and north-south axes radiating from Fargo, ND by Dr. Diane Manthey (Manthey, 1984). All collections were stored at 4°C, 43% relative humidity, in the long term seed storage facility, Agronomy Hall, Iowa State University.

Sample preparation

Seeds from each accession were randomly selected from within bulk samples and germinated in a greenhouse under uniform environmental conditions (16 h light:8 h dark, 25°C, light intensity about 700 $\mu M\ m^{-2}\ s^{-1}$). The tissue extraction protocol was adapted from Wendel and Weeden (1989). After seedlings reached the 3-4 leaf stage, about 8 plants per accession (range 1-20) were harvested and ground in an extraction buffer (50 mM phosphate buffer at pH 7.5, with 5% sucrose and 0.1% fresh mercaptoethanol), centrifuged and then either immediately loaded on gels, or stored frozen at -80°C. All steps were conducted under cold conditions (on ice or in a refrigerated chamber at 4°C) to preserve enzyme activities.

Gel electrophoresis

A three gel, thirteen enzyme system for foxtails was established after extensive testing of tissue age, tissue type, extraction buffer, tissue:buffer ratio, gel buffer, wattage and duration of electrophoresis, and staining procedures. The thirteen enzymes used were Aconitate Hydratase (ACO), Adenylate Kinase (ADK), Aldolase (ALD), Aspartate Aminotransferase (AAT), Diaphorase (DIA), Glutamate Dehydrogenase (GDH), Isocitrate Dyhydrogenase (IDH), Malate Dehydrogenase (MDH), Phosphogluconate Dehydrogenase (PGD), Phosphoglucose Isomerase (PGI), Phosphoglucomutase (PGM), Shikimate Dehydrogenase (SKD), and Triose Phosphate Isomerase (TPI). The three gels were C gel, D gel and CT gel, representing different gel buffers and electrode buffers. Details about these buffer systems and enzyme staining techniques can be found in Wendel and Weeden (1989).

Gel interpretation and data analysis

Following staining, zymograms were scored for individual genotypes in each accession. Knowledge of the number of loci involved, enzyme subunit structure and subcellular location in other species made it possible to interpret the zymograms in the foxtails (Weeden and Wendel, 1989). The loci were numbered sequentially, with the most anodal designated as 1. Within a locus, the alleles were assigned A, B, C, and so on, based on allele frequencies or the sequence of being detected during the study period, in decreasing order.

Genotypes were scored and genotypic data were analyzed with the computer software program BIOSIS-1 (Swofford and Selander, 1981). Allelic frequencies were generated for each accession. Calculation of allele frequencies for a geographic region was accomplished by taking the unweighted arithmetic means of population frequencies within that region. Nei's genetic identity between regions was based on the regional population allele frequencies (including monomorphic loci). Allelic frequencies were used to calculate genetic diversity statistics for geographic regions. They included the number of unique alleles, percentage of polymorphic loci (P), mean number of alleles per locus (A) and mean panmictic heterozygosity (H). Also calculated were parameters partitioning total genetic diversity into within- and between-population components in order to assess population differentiation. Total genetic diversity (H_T) was divided into a within-population component (H_S), and a between population component (D_{ST}; Nei, 1987). The ratio of D_{ST}:H_T, or G_{ST}, measured the extent of population differentiation. For principal component analysis, the allelic frequencies of polymorphic loci were transformed, with the computer software program NTSYS (Rohlf, 1991), into a inter-allele variance-covariance matrix, from which principal components were extracted. The first two principal components, accounting for a major share of the total variance, were projected onto a two dimensional plane to provide a graphic display of the interrelationships among accessions.

Results

One hundred sixty-eight S. viridis accessions were screened for thirteen enzymes, encoded by at least 28 loci. Out of the 28, 18 were polymorphic (no

criterion) so that the P = 64.3% (Table 2, 3). If judged by the 0.95 criterion, P decreased to 25%. Genetic polymorphism was rather weak, with common alleles typically having frequencies over 0.95 for the majority of loci. Averaged across all loci, S. virldis as a species had a H value of 0.0696 and A = 1.86. To estimate the degree of population differentiation, genetic diversity was further partitioned into within- and between-population components (Table 4). The loci ACO2, PGD1, SKD1 were the most variable, and ACO1, PGD3, TPI1, and TPI2 were the least variable. The values of G_{ST} were generally high across all loci, except for TPI1 and TPI2. Overall, S. viridis had an average G_{ST} value of 0.6468. The multi-locus genotype most frequently observed occurred in 25 accessions, and was found in Belgium, Canada, Czech, Japan, Turkey, and US (IN, ND, MD, MN, and WA). Its allelic composition was AAT1-A, AAT2-A, ACO1-A, ACO2-B, DIA1-A, DIA2-A, IDH3-C, MDH1-A, MDH2-A, MDH4-A, PGD1-B, PGD3-A, PGI1-A, PGI3-A, PGM1-A, SKD1-A, TPI1-A and TPI2-A.

The genetic diversity and its geographic patterns were further analyzed at various geographic levels. At the continental level, North American accessions (total of 127) were dispersed across the PCA1 axis to the same extent as the Eurasian accessions (total of 41). This complete overlap suggested the degree of population differentiation within each region was the same and there was no genetic differentiation between the two regions (Figure 1). Further analysis (not presented) showed that the Nei's unbiased genetic identity between the two regions was 1.0. Secondly, North American accessions dispersed along both PCA axes while Europe and Asian accessions, overlapping with each other, had mostly negative PCA2 scores. In other words, there were both similarities and differences in the patterns of genetic differentiation among accessions from Asia, Europe and North America. Statistically, North American and Eurasian accessions were nearly equal in genetic diversity but the latter had more unique, rare alleles, as reflected in the large decrease of P vaiues from the "no criterion" to "95% criterion", with a large A but similar H estimates (Table 2, 3). Alleles unique to North America were AAT1-B and TPI2-B (AAT1-B was not always stainable and it could actually occur in both groups in higher frequencies). Eurasian accessions had 10 unique alleles: AAT2-B, ACO-1B, DIA2-B, MDH1-B, MDH2-B, PGM1-B, PGM1-C, SKD1-C, TPI1-B, TPI2-C. In addition, the North

Table 2. Allele frequencies by regions (unweighted arithmetic mean of population frequencies); (number), number of populations; N. A.: North America

Loci	Allele	Southern. N. A. (51)	Northern N. A. (76)	Eurasia (41)	N. A. (127)	S. viridis (168)
AAT1	A	0.9886	0.9213	1.0000	0.9483	0.9609
	B	0.0114	0.0787	0.0000	0.0517	0.0391
AAT2	A	1.0000	1.0000	0.9744	1.0000	0.9940
	B	0.0000	0.0000	0.0256	0.0000	0.0060
ACO1	A	1.0000	1.0000	0.9816	1.0000	0.9957
	B	0.0000	0.0000	0.0184	0.0000	0.0043
ACO2	A	0.2135	0.2802	0.0138	0.2534	0.1948
	B	0.7865	0.7198	0.9862	0.7466	0.8052
DIA1	A	0.9804	0.9868	0.8872	0.9843	0.9619
	B	0.0196	0.0132	0.1128	0.0157	0.0381
DIA2	A	1.0000	1.0000	0.9718	1.0000	0.9935
	B	0.0000	0.0000	0.0282	0.0000	0.0065
IDH3	A	0.0216	0.0602	0.0493	0.0447	0.0452
	B	0.0000	0.0132	0.0110	0.0079	0.0145
	C	0.9784	0.9266	0.9397	0.9474	0.9403
MDH1	A	1.0000	1.0000	0.9705	1.0000	0.9932
	B	0.0000	0.0000	0.0295	0.0000	0.0068
MDH2	A	1.0000	1.0000	0.9705	1.0000	0.9932
	B	0.0000	0.0000	0.0295	0.0000	0.0068
MDH4	A	0.8889	0.9984	0.8836	0.9544	0.9385
	B	0.0131	0.0000	0.1053	0.0053	0.0284
	C	0.0980	0.0016	0.0111	0.0404	0.0331
PGD1	A	0.8462	0.0991	0.3210	0.3991	0.3762
	B	0.1077	0.8677	0.5667	0.5625	0.5687
	C	0.0461	0.0332	0.1123	0.0384	0.0551
PGD3	A	1.0000	0.9961	0.9915	0.9976	0.9962
	B	0.0000	0.0039	0.0085	0.0024	0.0038
PGI1	A	0.9348	0.8596	0.8821	0.8898	0.8893
	B	0.0652	0.1404	0.1179	0.1102	0.1107
PGI3	A	0.8625	0.9697	0.9987	0.9267	0.9443
	B	0.1375	0.0303	0.0013	0.0734	0.0558
PGM1	A	1.0000	1.0000	0.9701	1.0000	0.9931
	B	0.0000	0.0000	0.0214	0.0000	0.0050
	C	0.0000	0.0000	0.0085	0.0000	0.0020
SKD1	A	0.4338	0.9806	0.8597	0.7610	0.7867
	B	0.5663	0.0194	0.1146	0.2391	0.2073
	C	0.0000	0.0000	0.0256	0.0000	0.0060
TPI1	A	1.0000	1.0000	0.9987	1.0000	0.9997
	B	0.0000	0.0000	0.0013	0.0000	0.0003
TPI2	A	0.9975	1.0000	0.9987	0.9990	0.9990
	B	0.0025	0.0000	0.0000	0.0010	0.0007
	C	0.0000	0.0000	0.0013	0.0000	0.0003

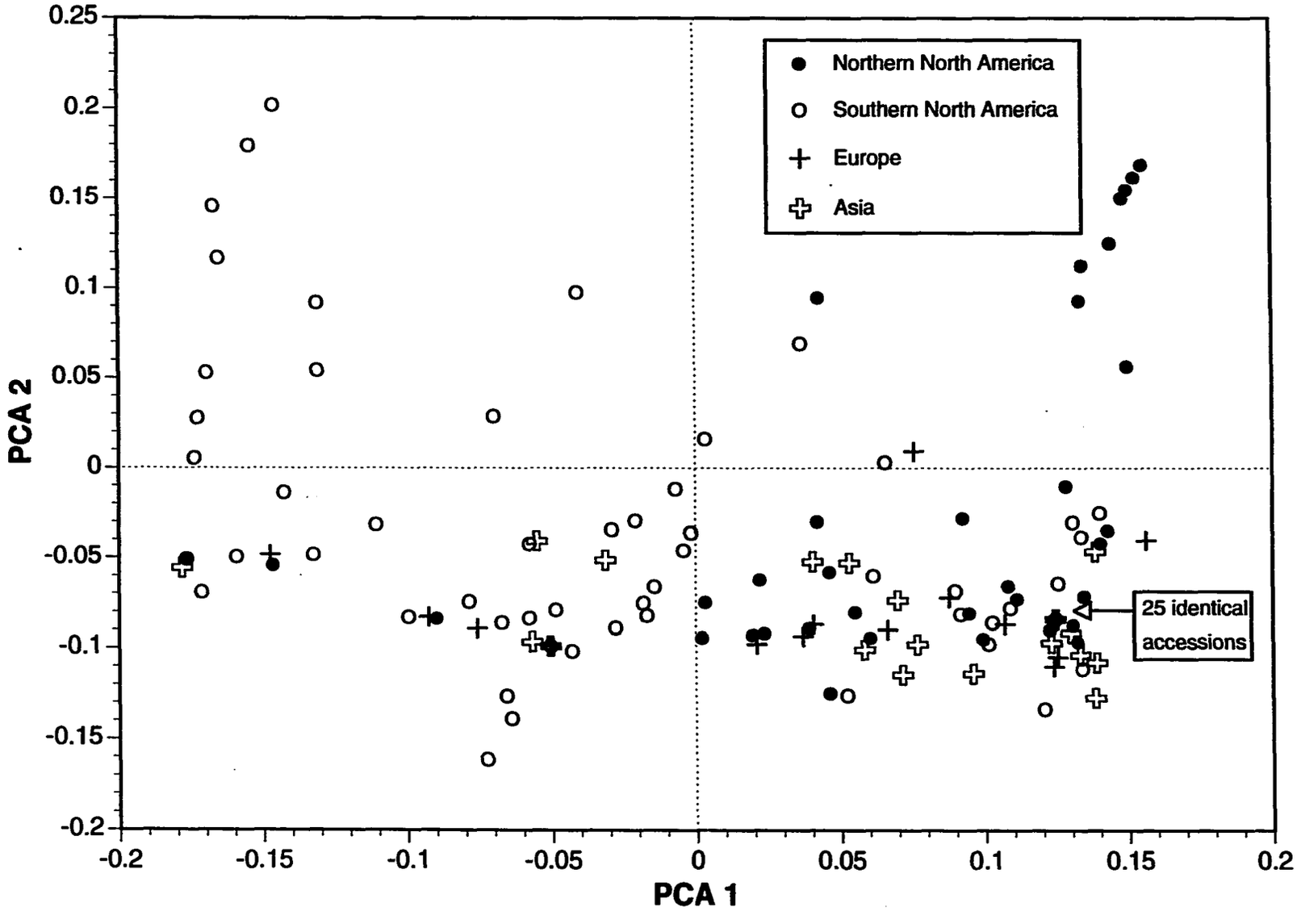
Table 3. Summary statistics of the genetic diversity at 28 loci in S. viridis (including four S. italica accessions) at the regional and species levels. Calculations were based on unweighted arithmetic means of population frequencies; number in parenthesis are number of accessions; N. A., North America

Parameters	Southern N. A. (51)	Northern N. A. (76)	N. A. (127)	Eurasia (41)	S. viridis (168)
No. of unique alleles	2	2	2	10	
% of Polymorphic Loci (No criterion)	35.7	35.7	39.3	60.7	64.3
% of Polymorphic Loci (0.95 criterion)	21.4	17.9	25.0	21.4	25.0
Mean no. of alleles per locus	1.43	1.43	1.50	1.79	1.86
Mean panmictic heterozygosity	0.063	0.046	0.069	0.068	0.070

Table 4. Partitioning of total genetic diversity (H_T) into within- (H_S) and between-population portions (D_{ST}) for S. viridis; coefficient of population genetic differentiation (G_{ST}) = D_{ST}:H_T

Loci	H_S	H_T	D_{ST}	G_{ST}
AAT1	0.0246	0.0751	0.0505	0.6726
AAT2	0.0000	0.0118	0.0118	1.0000
ACO1	0.0032	0.0085	0.0053	0.6224
ACO2	0.0614	0.3137	0.2522	0.8041
DIA1	0.0029	0.0733	0.0704	0.9610
DIA2	0.0054	0.0130	0.0077	0.5882
IDH3	0.0251	0.1135	0.0884	0.7786
MDH1	0.0051	0.0136	0.0085	0.6257
MDH2	0.0051	0.0136	0.0085	0.6257
MDH4	0.0182	0.1173	0.0991	0.8446
PGD1	0.1167	0.5320	0.4153	0.7807
PGD3	0.0051	0.0075	0.0024	0.3148
PGI1	0.0475	0.1968	0.1493	0.7586
PGI3	0.0210	0.1053	0.0843	0.8007
PGM1	0.0069	0.0138	0.0069	0.4971
SKD1	0.0625	0.3380	0.2755	0.8151
TPI1	0.0006	0.0006	0.0000	0.0497
TPI2	0.0019	0.0021	0.0002	0.1028
Mean	0.0230	0.1083	0.0854	0.6468
Mean including 10 invariant loci	0.0148	0.0696	0.0549	---

Figure 1. PCA plot of isozyme variation of 42 alleles in 168 S. viridis accessions (including four S. italica) from Asia, Europe and North America. PCA1 counts for 31% of total variance and PCA2 18%. Legend: ● Northern group (North America), o Southern group (North America), + Europe, ✣ Asia.


Northern North America
Southern North America
Europe
Asia
25 identical accessions
PCA 1
PCA 2
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.2
-0.15
-0.1
-0.05
0
0.05
0.1
0.15
0.2

American group had a high frequency at ACO2-A, while Eurasian accessions had high frequencies at DIA1-B, MDH4-B and PGD1-C.

Within North America, accessions could be roughly divided into a Southern group and a Northern group along a line at 43.5°N latitude: those to the north had positive scores on PCA1, and those to the south had negative scores on PCA1 (Figure 1). The Northern type was less variable than the Southern type. These two groups had a genetic identity of 0.97, and shared the same number of unique alleles and number of alleles per locus. The Southern group, however, had higher P (0.95 criterion) and H, indicating its slightly higher genetic diversity. Two alleles unique to the Southern group were MDH4-B and TPI2-B. The alleles unique to the Northern group were IDH3-B and PGD3-B. In addition, the Southern group had high frequencies of PGD1-A and SKD1-B, whereas the Northern group had high frequencies of PGD1-B and SKD1-A.

Within Europe, no consistent geographic pattern in genetic diversity was found. Within Asia, accessions from China and Japan had positive scores in PCA1. Turkish accessions were scattered along the entire PCA1 axis (not presented).

At the state, county and farm level, the degree of genetic differentiation showed little hierarchical patterning. Accessions from Iowa (mostly central and northeastern) had extensive genetic differentiation, comparable to that found in the entire collection used in this study (Figure 2). Those from the Fargo, ND area, however, were relatively homogeneous, typical of the Northern cluster in North America. At the farm level, differentiation could be either strong (e. g., farm 2, 3) or weak (e. g., farm 1, 5). Differentiation at the Luther, IA farm was intermediate, but one rare allele, MDH4-C, was found there.

The systematic relationships between green foxtail and foxtail millet, and among several green foxtail varieties, were also examined. The allozyme markers of foxtail millet and other green foxtail varieties did not differ greatly from common green foxtail (Figure 3). Indeed, only one unique diagnostic allele occurred in foxtail millet and none was found in the other green foxtail varieties.

To aid future studies of weedy adaptation in Setaria spp., several genetically divergent accessions were selected to comprise a model system (test array) of S. viridis diversity.

Figure 2. PCA plot of isozyme variation of 42 alleles in S. viridis accessions assembled at various levels of geographic range (Same PCA as in Figure 1 with only North American accessions shown). PCA1 counts for 31% of total variance and PCA2 18%. All farms are located in Iowa. Legend: 1 = Holy Cross farm, 2 = Washington farm, 3 = Decorah farm, 4 = Luther farm, 5 = Ames farm, • North America except Iowa and North Dakota.

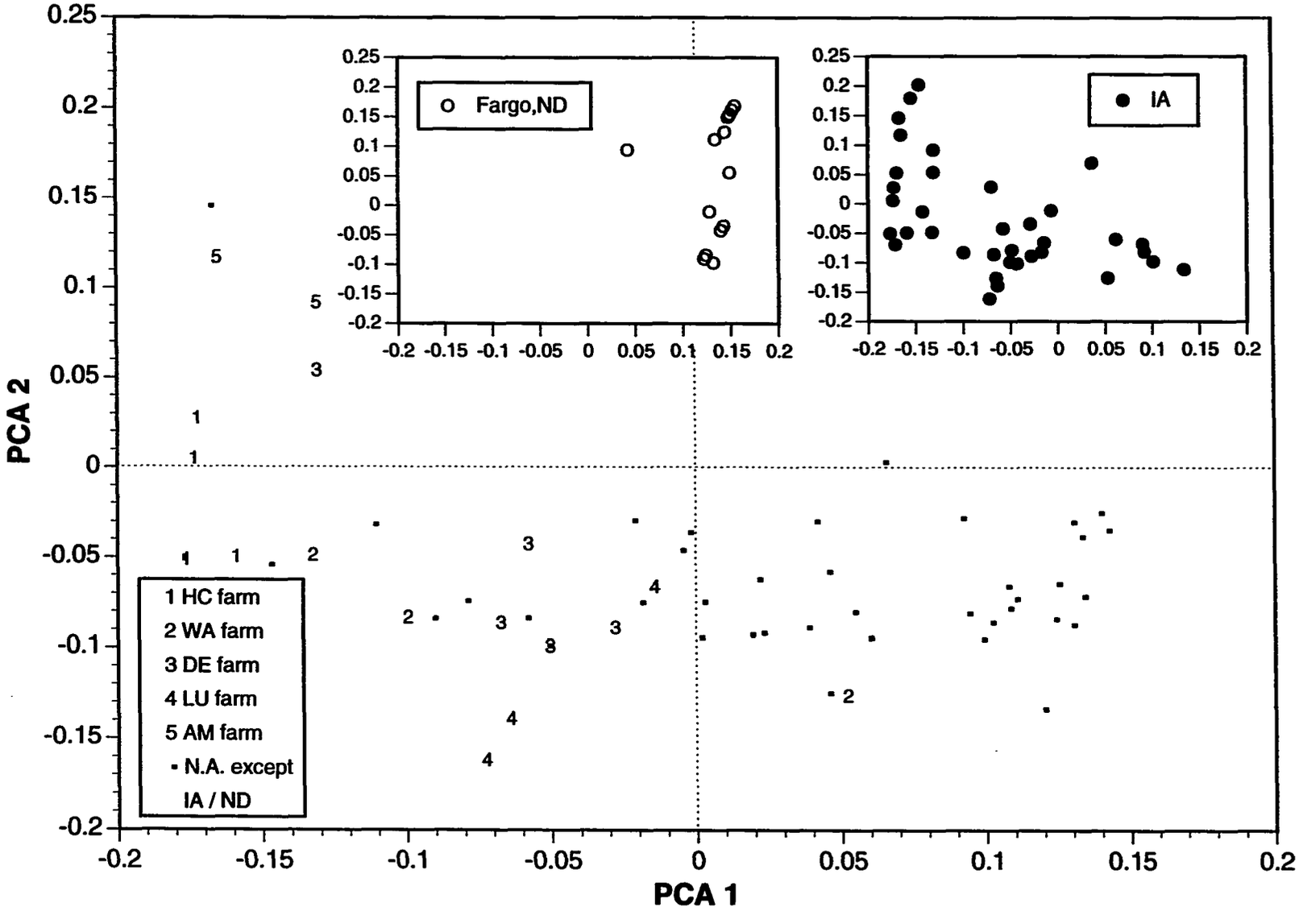
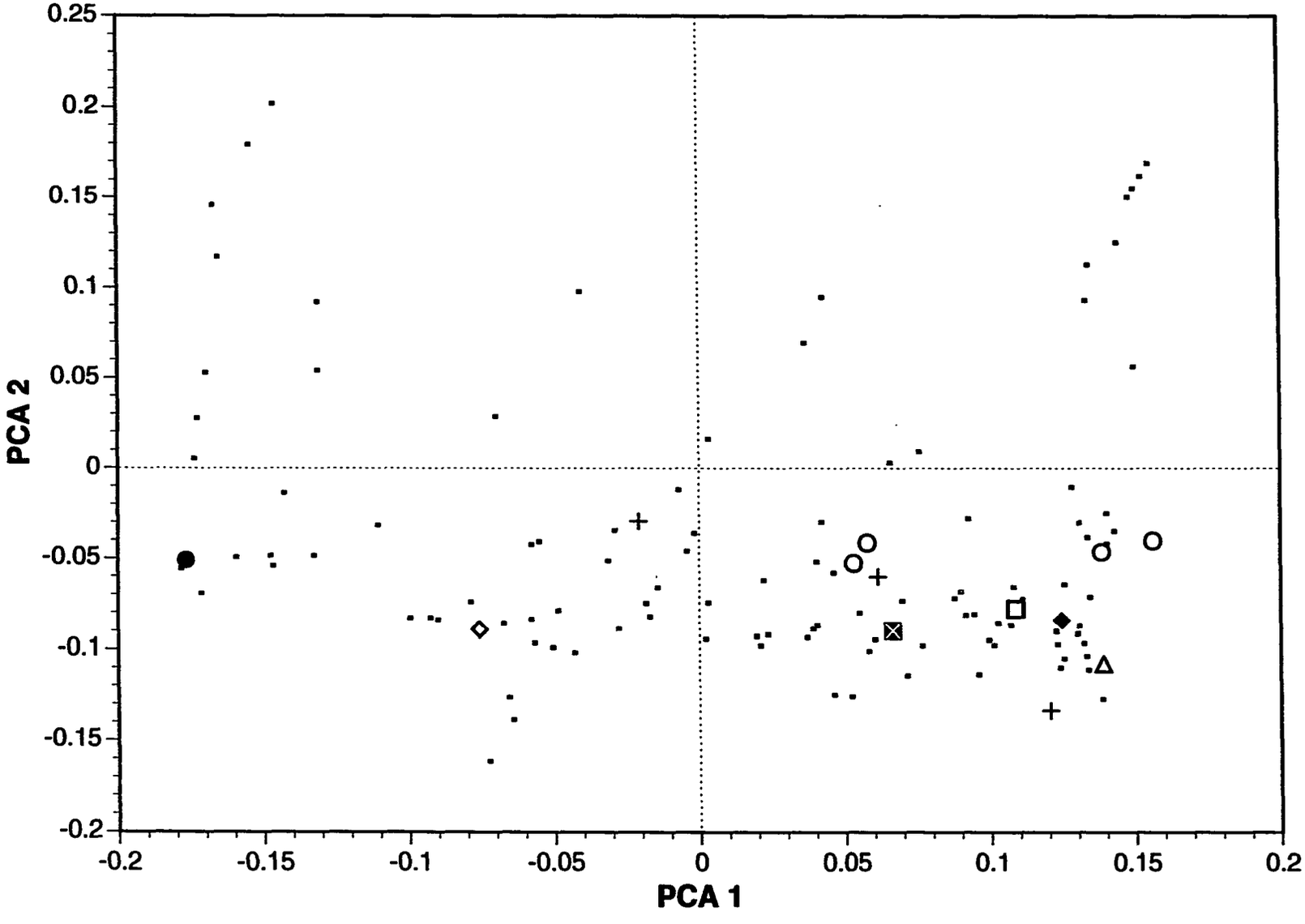

PCA 2
PCA 1
Fargo,ND
IA
1 HC farm
2 WA farm
3 DE farm
4 LU farm
5 AM farm
N.A. except IA / ND

Figure 3. PCA plot of S. italica, various S. viridis varieties, and a triazine-resistant biotype (Same PCA as in Figure 1 with all 168 accessions shown). PCA1 counts for 31% of total variance and PCA2 18%. Legend: ▪ var. viridis, ● triazine-resistant biotype, + var. major, ◆ var. robusta purpurea, □ var. robusta alba, O S. italica, Δ var. pachystachys, ◇ var. weinmanni, ⊠ var. pycnocoma.


0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
PCA 2
-0.2
-0.15
-0.1
-0.05
0
0.05
0.1
0.15
0.2
PCA 1

Discussion

Genetic diversity, structure and weedy adaptation

Green foxtail's genetic diversity and population genetic structure provide important clues about relationships among its populations from around the world. Of primary concern also is the taxonomic status of foxtail millet, green foxtail and its several varieties.

Genetic diversity Overall, green foxtail was low in total genetic diversity but high in population genetic differentiation compared to other plant species. Genetic diversity and its partitioning in plant species have been extensively reviewed in Hamrick and Godt (1990): an average plant species has a P of 50% (criterion unknown), A of 1.96, and H of 0.15; at the population level, P, A and H are 34%, 1.53 and 0.11 respectively; and the average G_{ST} was 0.22. In comparison, green foxtail had P = 25% (0.95 criterion), A = 1.86 (no criterion) and H = 0.07 (Table 3). When total genetic diversity was further partitioned into within and between population components to measure the degree of population differentiation, about 65% of the total variation was that found between populations (Table 4).

Table 5 was compiled from previous isozyme studies of genetic diversity in several self-pollinating weed species. Many of these reports had incomplete statistical analyses. Data for these parameters for green foxtail were generally equivalent to those self-pollinating weeds. For instance, green foxtail had a H value of 0.07, compared to the H of 0.06 in Eichhornia paniculata and H of 0.01 in Bromus tectorum. Green foxtail also had a A of 1.86 whereas Echinochloa crus-galli had a A of 1.81. The G_{ST} of 0.65 in green foxtail was within the G_{ST} range of 0.33 - 0.8 reported in these weeds. Overall, introduced self-pollinating weeds are low in total genetic diversity but exhibit strong population differentiation (Barrett, 1988; Barrett and Richardson, 1986; Barrett and Shore, 1989; Brown and Marshall, 1981; Rice and Jain, 1985). In an earlier study (Jusuf and Pemes, 1985; Table 5), genetic diversity statistics reported for S. viridis were higher than those from our studies, as well as higher than those of other self-pollinating weeds. Of the five enzymes used in Jusuf and Pernes' study, esterase and acid phosphatase were difficult to interpret because they have less well defined biochemical functions, and their number of expressed

Table 5. Genetic diversity in some predominantly self-pollinating weed species; n = number of accessions surveyed; P, % of polymorphic loci; A, mean number of alleles per locus including monomorphic loci; H_T, mean panmictic heterozygosity including monomorphic loci; G_{ST}, coefficient of genetic differentiation among populations. Numbers in brackets were estimates based on the information provided. [1]average population values; [2]regional genetic diversity estimates (France and China). Criteria for polymorphism were unknown unless otherwise noted

Taxon	n	Locl	P	A	H_T	G_{ST}	References
Abutilon theophrasti	39	27	7				Warwick, 90
Bromus tectorum[1]	60	25	4.6 *0.99*	1.05	0.01	0.48	Novak et al, 91
Datura stramonium	9	22	0				Warwick, 90
Echinochloa phyllopogon	12	25	[28]	[1.32]		0.80	Barrett,88
Echinochloa crus-galli	11	31	[52]	[1.81]		0.33	Barrett,88
Echinochloa oryzoides	12	32	[16]	[1.16]		0.50	Barrett,88
Eichhornia paniculata	5	21	7.6[1]		0.06	0.57	Barrett & Shore,89
Panicum miliaceum	39	19	5				Warwick, 90
Pyrrhopappus carolinianus	4	13	25 *0.99*	1.29	0.07	0.47	Petersen et al.,90
Setaria faberii	8	24	12				Warwick, 90
Setaria viridis[2]	45	10	[80] *0.95*	[2.2]	0.12- 0.18		Jusuf & Pernes, 85
Sorghum halepense	13	21	14				Warwick, 90

loci, enzyme subcellular compartmentation and subunit structures were less conserved (Weeden and Wendel, 1989). However, it was also possible that the green foxtail populations from France and China examined in that study were genetically more diverse than the germplasm we analyzed herein.

Patterns of genetic diversity There were both similarities and differences in the patterns of genetic differentiation among accessions from Asia, Europe and North America. North American and Eurasian S. viridis accessions had equivalent genetic diversity and within-region population differentiation (Table 3, Figure 1). Genetic differentiation between the two regions was relatively weak with the genetic identity of the two regions equalling 1.0. The Eurasian accessions had more unique alleles than did the North American accessions.

North American accessions, at a macroscale, could be roughly divided into two groups separated at 43.5° N latitude (e. g. Minnesota-Iowa border). The southern North American group was slightly higher in genetic diversity than the northern group. The genetic identity between the two was 0.97. In other words, the genetic differentiation within North American green foxtail accessions was slightly greater than that observed between North American and Eurasian accessions. A similar intraregional differentiation was also observed between accessions from France and China within Eurasia (Jusuf and Pernes, 1985). Among our Eurasian accessions, however, no geographic pattern in genetic diversity was observed.

The size of the geographic range from which populations were sampled was not an accurate indicator of the extent of genetic diversity found among populations from that region. This generality can be seen among accessions taken from within regions of various sizes: Manitoba (not shown), Iowa, the area around Fargo, ND, and from individual farms. The genetic diversity of green foxtail populations from a particular local geographic area was probably largely determined by both the number of independent introductions to that area, and the intensity and duration of natural selection pressures at those sites. At the regional level, accessions from the state of Iowa were relatively genetically diverse, nearly as diverse as the total sample in this study (Figure 2). In general, Iowa populations were primarily from the Southern cluster in North America, but possessed many from the Northern cluster too. In contrast, accessions from

Manitoba were tightly clustered, with low diversity (data not shown). In the more restricted, county-sized, area around Fargo, ND, little differentiation occurred (Figure 2). At the microscale of individual farms, populational diversity was impossible to predict. For example, in five Iowa farms, green foxtail populations could be genetically either very similar or relatively well differentiated. The most widely distributed green foxtail genotype occurred in 25 accessions from six countries, from both the Old World and the New World (Figure 1). Its allelic composition at 18 loci were fixed in these accessions.

Normally, introduced weed populations should have reduced genetic diversity because of the founder effect, by which they represent only a subsample of the original gene pool. To a certain degree, the founder effect was observed in S. viridis. The accessions from North America had reduced allelic richness compared to those of Eurasia: fewer unique alleles, lower percentage of polymorphic loci and fewer alleles per locus (Table 3). Genetic drift probably has occurred in S. viridis, as indicated by the many fixed alleles in North American accessions (Table 2). On the other hand, heterozygosity was equal between Eurasian and North American accessions and Nei's genetic identity between them was 1.0. A possible explanation was that there were multiple introductions of green foxtail from the Old World to the New World. Prehistoric migration of Setaria could date from when the ancestors of contemporary native Americans first crossed the Bering Strait ca. 8000-10,000 years ago. Foxtail migration and dissemination were also facilitated by the large human immigration from the Old World to North America in historical times, as well as the high volume of international grain trade in modern times. One such example was S. faberii. This species was reported to have arrived in the US in 1920's as a grain contaminant from China (Rominger, 1962). By 1970's, it expanded into Canada (Warwick, 1990). In little over a half century, it has been distributed over a large part of North America (Hafliger and Scholz, 1980).

There are several interpretations for green foxtail population genetic structure in North America. It may be a result of multiple introductions, followed by adaptation and differentiation due to natural selection in its new range. Multiple introductions of green foxtail, in the absence of local adaptation, should have produced a random, mosaic pattern of geographic distribution amongst

North American accessions. Instead, we observed a north-south regional differentiation amongst populations. Such a regional divergence suggests that since their several introductions natural selection has led to locally adapted foxtail genotypes. Populations with genotypes more suitable to northern conditions probably flourished there, while those more suited to southern conditions thrived in the south. Possibly the more favorable growing conditions (rainfall, length of season, temperature) of the southern part of North America lead to their greater diversity relative to that in the north. An alternative interpretation for green foxtail population genetic structure in North America is that there is a North-South gradient in Eurasian populations. During green foxtail introduction to the New World, the same gradient was also duplicated in North America. This hypothesis can not be addressed in this study due to inadequate sampling in Eurasia, particularly in its southern region. The third interpretation is that there occurred parallel development of this North-South gradient in both the Old World and the New World.

Weedy adaptation The population genetic structure of many introduced, self-pollinating, weed species clearly indicates that a high level genetic variation is not a prerequisite for successful colonization (Barrett and Shore, 1989). Fox example, several important agricultural weeds, Abutilon theophrasti, Panicum miliaceum and Sorghum halepense have very little genetic polymorphism as revealed by their isozymes (Table 5). Two contrasting strategies have been proposed to explain weedy adaptation (Baker, 1965, 1974; Bradshaw, 1965; Barrett and Richardson, 1986). One strategy relies on genetic polymorphism for the development of locally adapted "specialist" genotypes. Another strategy relies on phenotypic plasticity for the development of "general purpose" genotypes adaptable to a wide range of environmental conditions.

Many green foxtail populations were genetically strongly differentiated, heterogeneous (e. g. northern and southern North America). Some populations remained identical. A common green foxtail genotype was found in many geographic locations, in very different ecological conditions: the midwestern U.S. Corn Belt areas of IN, MN, and ND; the eastern U.S. Corn Belt of MD; the western U.S. agricultural valley of WA; the highlands of Ontario, Canada; the lowlands of Belgium in N.W. Europe; the plains of Bohemia, Czechoslovakia in

central Europe; the waste areas interspersed between limestone outcroppings in the Akiyoshi Dai National Park, Honshu, Japan; and between cracks in a cement pier over the bay in Yokohama Harbor, Honshu, Japan. Interestingly, this common genotype has not yet been found in Iowa, despite the diversity of green foxtail populations found in this state. Such a population genetic structure of green foxtail suggests that the species may possess both generally-adapted and specially-adapted genotypes. To test this hypothesis in future studies, several accessions were selected to comprise a model system (test array) representing S. viridis diversity. Seven divergent genotypes (accessions 843, 1144, 1219, 1266, 1278, 1693, 1801) were selected to test the degree of specialization in adaptive traits. A common genotype (e.g. accession 1604) found in 25 different populations was selected to test the degree of general adaptation by a single genotype to a range of environmental conditions.

Taxonomic status of S. italica and some green foxtail varieties

Views about the origin of S. italica (L.) Beauv., its domestication, and its taxonomic relationship to green foxtail, S. viridis, have varied. Linnaeus (1753) was the first to recognize foxtail millet and green foxtail as two independent species, Panicum italicum and P. viride. Beauvois (1812) later transferred both into the genus Setaria. Ascherson and Graebner (1899) reduced the status of foxtail millet to a variety under green foxtail as Panicum viride B. P. var. italicum. Briquet (1910) later reclassified foxtail millet as a subspecies of green foxtail, S. viridis ssp. italica. Evidence in support of this subspecies classification includes their morphological similarity, the ease of hybridization between the two resulting in fertile progeny and a lack of qualitative morphological differences between the two taxa (Rominger, 1962). Other evidence for this classification is that populations of the two taxa derived from the same region have stronger genetic similarities than that found within the same species derived from different regions (Darmency and Pernes, 1987; De Wet et al., 1979; Jusuf and Pernes, 1985; Li et al., 1945; Nguyen and Pernes, 1985; Till-Bottraud et al., 1992; Vishwanatha et al., 1981). Reviewing this evidence, Prasada Rao et al. (1987) suggested that both taxa be considered as subspecies of S. italica.

Allozyme data generated herein for the four accessions of S. italica demonstrated that: 1) foxtail millet contained only one unique diagnostic allele;

and 2) it was completely embedded amongst the green foxtails accessions on the PCA plot. These findings support the interpretation that S. italica is a domesticated form of weedy green foxtail and further bolsters the broader taxonomic concept of S. viridis being composed of both the weedy and domesticated foxtails. A particularly interesting question that our allozyme data do not allow us to address is whether the foxtail millets were domesticated once, with subsequent dispersal and radiation, or several times, independently, from different S. viridis sources.

Green foxtail varies greatly in morphology and growth characteristics. A number of such variants have been previously considered as varieties without knowledge of their genetic composition. The named varieties studied herein included S. viridis var. viridis; S. viridis var. weinmanni (R. & S.) Brand.; S. viridis var. major (Gaud.) Posp.; S. viridis var. robusta-alba Schreiber; S. viridis var. robusta-purpurea Schreiber; S. viridis var. pycnocoma; S. viridis var. pachystachys (Figure 3). We observed that these varieties were completely embedded in common green foxtail, S. viridis var. viridis, on PCA plots. Because they show no genetic differentiation from the common type, the validity of their classification as distinct taxonomic varieties is questioned.

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WEEDY ADAPTATION IN SETARIA SPP.: II. : ISOZYME ANALYSIS OF GENETIC DIVERSITY AND POPULATION GENETIC STRUCTURE IN S. GLAUCA, S. GENICULATA, and S. FABERII

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Rong Lin Wang, Jonathan Wendel and Jack Dekker

Abstract: Setaria glauca (yellow foxtail), S. geniculata (knotroot foxtail) and S. faberii (giant foxtail) are important cosmopolitan weeds of temperate and tropical areas of the world. Isozyme markers were used to investigate the genetic diversity and population genetic structure in 94 accessions of yellow foxtail, 24 accessions of knotroot foxtail, and 51 accessions of giant foxtail collected mainly from North America and Eurasia. The genetic diversity and population genetic structure in these species provided important clues about their evolutionary history. Compared to an average plant species, yellow and knotroot foxtail had low genetic diversity and marked population differentiation. In yellow foxtail, the percentage of polymorphic loci was 13.5% (0.95 criterion), mean number of alleles per locus was 1.54 (no criterion), mean panmictic heterozygosity was 0.042, and coefficient of population genetic differentiation was 0.73. In knotroot foxtail, they were 18.9%, 1.38, 0.096 and 0.91 respectively. Yellow foxtail and knotroot foxtail were genetically closely allied, with a Nei's genetic identity of 0.83. There were geographic patterns in the genetic diversity of both species. For both, genetic divergence between those accessions from Eurasia and North America was minimal. Populations in their native range had slightly greater genetic diversity than those from the introduced range. Yellow foxtail populations separated into an Asian cluster, European cluster and North American cluster. Within North America, Iowan yellow foxtail populations were genetically diverse whereas all populations collected from other continental locations were essentially identical. Knotroot foxtail populations in North America were genetically differentiated along a line at about 37°N latitude into a northern and southern group. No geographic pattern of genetic diversity was observed in knotroot foxtail populations from Eurasia.

The current yellow foxtail population genetic structure probably resulted from a variety of factors: number of historical introductions, environmental heterogeneity and natural selection, intraspecific variations in ploidy levels and reproductive isolation, and biased sampling. The population genetic structure of knotroot foxtail in the U.S. may have been shaped by natural selection. The giant foxtail populations studied were essentially genetically identical. Of the 51 accessions of giant foxtail screened, only one population had evidence of isozyme polymorphism.

Introduction

Like S. viridis considered in an earlier companion study (Wang et al., 1994), Setaria glauca (yellow foxtail), S. geniculata (knotroot foxtail), and S. faberii (giant foxtail) are also important weeds. Yellow foxtail and giant foxtail are both annuals, and knotroot foxtail is a rhizomatous perennial. In many areas of North America, two or more of these four weedy foxtail species are often found growing together in individual agricultural fields.

Neither the evolutionary origin of, nor the systematic relationships between, the weedy foxtails are well understood. Yellow foxtail and knotroot foxtail are genetically closer to each other than they are to S. italica, a likely subspecies within green foxtail (Chikara and Gupta, 1980; Wang et al., 1994). Previous studies suggested that giant foxtail was derived from green foxtail (Li et al., 1942, 1945). Yellow, giant and knotroot foxtails are all polyploids, a trait often considered derived from a diploid state like that in green foxtail (Rominger, 1962). Knotroot foxtail is considered less advanced than yellow foxtail because the former is a rhizomatous perennial (Rominger, 1962). Both yellow foxtail and knotroot foxtail include populations with different chromosome numbers; the karyological variants are nevertheless lumped into one taxon. Yellow foxtail chromosome numbers might be 2n = 36, 2n = 72, 2n = 18, 2n = 44 and aneuploids, with 2n = 36 and 2n = 72 most common (Rominger, 1962; Steel et al., 1983; Khosla and Sharma, 1973). Knotroot foxtail chromosome numbers can be either 2n = 36 or 2n = 72. Giant foxtail's chromosome number is 2n = 36 (Pohl, 1962). All three species

exhibit highly variable morphology, growth, and developmental characteristics (Rominger, 1962; Steel et al., 1983).

Giant foxtail probably originated in China (Pohl, 1951), whereas yellow foxtail is native to Eurasia. Knotroot foxtail is considered to be a native of either Central America or northern South America (Rominger, 1962). Although yellow and knotroot foxtails probably originated on different continents, they are morphologically quite similar and are easily confused with each other. Knotroot foxtail is also closely related to several Setaria species in tropical Africa, the suspected center of origin for the genus (Stapf and Hubbard, 1930; Rominger, 1962).

Today, all three species are widely distributed around the world (Hafliger and Scholz, 1980). Yellow foxtail occurs in North America, Argentina and Uruguay, southern and northern Africa, the Middle East, Europe, Asia, Australia and many Pacific islands. Yellow foxtail occurs throughout the U.S. as does green foxtail. Knotroot foxtail occurs throughout the Americas, southern Africa, most of Europe, the Middle East, Asia, Australia and several Pacific islands. In the U.S., knotroot foxtail occurs along the Atlantic coast from Massachusetts to Florida, the Gulf coast from Florida to Texas, and the Carolinas westward to Kansas and California (Rominger, 1962). Giant foxtail is found in central Europe, Russia, the Middle East and east Asia. In the U.S., giant foxtail is limited to the east, southeast and parts of the North Central region. All three foxtails are of significant agronomic importance, resulting in substantial crop yield losses and annual weed control expenditures. This Setaria spp. complex (including green foxtail) is considered one of the most serious weed groups in the U.S. and the world.

Biological diversity, wide geographic distribution, and strong competitiveness in disturbed habitats all mark foxtails as highly successful weeds. There is, however, little knowledge of what traits lead to their success as weeds, or what the implication and significance of foxtail genetic heterogeneity has in their adaptation. Understanding weedy adaptation is a key to developing more efficient, site-specific, weed management systems. Achievement of this goal will require a combination of new information about the population biology and physiology of the foxtails. An important aspect of

population biology is population genetic structure, which is poorly understood in foxtails.

Our objectives for this study are to investigate genetic diversity in yellow, knotroot and giant foxtail; to understand the geographic patterns of this genetic diversity at the continental, state and local levels; and to assess the genetic relationship between yellow and knotroot foxtail.

Materials and Methods

Seed collections

A total of 94 S. glauca, 24 S. geniculata and 51 S. faberii accessions were acquired for this project either through our own collection or from colleagues (Table 1, 2, 3). The collections came from 13 countries on three continents: Asia, Europe, and North America. Most foreign accessions were collected by one of us (JD) while other accessions were provided to us bythe U.S.DA-ARS North Central Regional Plant Introduction Station, Ames, IA. Within North America, accessions originated from 11 to 14 states depending on the species, but mostly from east of the Rocky Mountains and west of the Appalachians. They originated from as far north as North Dakota, and as far south as Texas and Louisiana, primary agricultural regions of the continent. In North America, Iowa and North Dakota were the most extensively sampled areas. The North Dakota accessions were systematically collected in agricultural fields adjacent to farm roads and highways about every three miles, along east-west and north-south axes radiating from Fargo, ND by Dr. Diane Manthey (Manthey, 1984). All collections were stored at 4°C, 43% relative humidity in the long term seed storage facility, Agronomy Hall, Iowa State University.

Sample preparation, gel electrophoresis, gel interpretation and isozyme data analyses were described in Wang, Wendel and Dekker (1994).

Results

Yellow foxtail

Genetic diversity in yellow foxtail was measured by percentage of polymorphic loci (P), mean number of alleles per locus (A), mean panmictic

Table 1. List of 94 S. glauca accessions. Latitude and longitude are the ranges or medians for the nation, provinces or states

Country and Region		Location	Accession #
ASIA			
China (19-53°N/74-134°E)	Heilongjiang	Harbin	628
		Jiamushi	1584
		Keshan	629
Japan (31-45°N/130-145°E)	Nagamo	Shinshu Univ.	2397
		unknown	1867
	Yamaguchi		2414
Turkey (36-41°N/26-44°E)		unknown	1863
Russia (50-78°N/25-180°E)		Birobidzan	2455
		Habarovosk	2452, 2456
		Vladivostock	2446
EUROPE			
Belgium (51°N/5°E)		unknown	1864
Czech (47-51°N/12-22°E)		Decin	2554
		Kosice	2494
	Lednice	Buclar-chodnik	2489
		Mirejovice	2524
		Prague	2505,2517
		Usti nad Labem	2529,2538
Germany (47-55°N/6-15°E)		Munich	2557
		Regensburg	2565
Russia (50-78°N/25-180°E)		Krasnoder	2470
		Moscow	2465, 2467-68, 2479
NORTH AMERICA			
Canada	Ontario(52°N/88°W)	Elora	4, 1483
		Markham	3
United States	AK (35°N/92°W)	Fisher	1748
	IA (42°N/94°W)	Allison	1220
		Ames	91, 98, 427
		Ankeny	1027
		Basset	904
		Blackhawk Co.	1217
		Blairsburg	906
		Delaware	1047
		Grundy Center	1222
		Hampton	1323
		Iowa Co.	1035
		Jasper Co.	1045
		Johnson Co.	1037
		Keokuk	1139
		Lansing	901
		Luther	1463
		Malcom	1039
		Marble Rock	903
		Maynard	1211
		Radcliff	907
		Readlyn	1213
		Tipton	1042
		unknown	1551
		Washington	1140
		Winnishiek Co.	1210
	IN (40°N/86°W)	unknown	1545

Table 1. (continued)

Country and Region		Location	Accession #
	KS (38°N/98°W)	Kansas St.Univ.	1695
	MD (38°N/76°W)	Beltsville	1274
	MN (46°N/92°W)	Rosemont	1620
		unknown	1619
	ND (47°N/100°W)	Colfax	1775-78
		Embden	1779-83, 1800
		Fargo	1613-17
		Hillsboro	1785-87, 1790
		unknown	1762
		Whapeton	1771-74, 1784, 1788-89
	PA (41°N/77°W)	Penn St Univ.	1629
		unknown	1542
	WA (47°N/120°W)	Prosser	1597
	WI (45°N/89°W)	Madison	1277
	WY (42°N/107°W)	Laramie	1701

Table 2. List of 24 S. geniculata accessions. Latitude and longitude are the ranges or medians for the nation, provinces or states

Country and Region		Location	Accession #
ASIA			
Afghanistan (30-39°N/61-71°E)		unknown	1860
		unknown	1861
India (9-36°N/70-96°E)		unknown	1865
Japan (31-45°N/130-145°E)		Takatsuki	2429
Osaka			
	Yamaguchi	Yamaguchi Univ.	2406
Turkey (36-41°N/26-44°E)		unknown	1862
		unknown	1866
Uzbekistan (41°N/69°E)		Tashkent	2466
Europe			
Italy (37-47°N/7-18°E)		Verona	2590
NORTH AMERICA			
United States	AK (35°N/92°W)	Clarkdale	1751
		Harrisburg	1747
	IN (40°N/86°W)	West Lafayette	1602
	KS (38°N/98°W)	unknown	1548
	KY (38°N/85°W)	Georgetown	1543
	LA (31°N/92°W)	Louisana State Univ.	1699
	MD (38°N/76°W)	Queenstown	1537
		unknown	1487
	MO (39°N/92°W)	unknown	1546
	NE (41°N/100°W)	unknown	1549
	OH (40°N/83°W)	unknown	1544
	TX (32°N/99°W)	Bushland	1702, 1703
	VA (38°N/78°W)	Blacksburg	1606
		unknown	1541

Table 3. A list of 51 S. faberii accessions

Country and Region		Location	Accession #
ASIA			
China	Heilongjiang	Jiamushi	1583
Japan		Omiya	2390, 2391
	Nagasaki	Kyushu Island	2403
	Yamaguchi	unknown	2415
		Sanmi Village	2418
	Kyoto	Kyoto	2434
Russia		Vladivostock	2448, 2449
		Harbarovsk	2451
EUROPE			
Russia		Moscow	2474, 2475
Czech		Usti nad Labem	2537, 2549, 2550
NORTH AMERICA			
Canada	Ontario	Blenheim	1482
Unite States	CA	Walnut Creek	1627
	IA	Ames	738
		Colo	1738
		Hampton	840, 841, 842
		Lansing	881, 884
	IL	unknown	1033
		unknown	1561
		Woodstock	25
	IN	unknown	1562
		West Lafayette	1600
	KS	Manhattan	1696
	KY	unknown	1564
	MD	Beltsville	1272, 1535
		Hartford Co.	1534
		Quantico	1538
		Queenstown	1536
		unknown	1486, 1488
	MI	Kalamazoo	1741, 1742
	MN	unknown	1567
		Rosemont	1621
		Waseca	1599
	MO	unknown	1560
	OH	unknown	1563
	PA	unknown	641, 1565
		Delta	1533
	VA	unknown	1566
		Blacksburg	1607
	WI	Madison	1276

heterozygosity (H), and the coefficient of population genetic differentiation (G_{ST}). Ninety-four accessions of yellow foxtail were screened for 13 enzymes, encoded by at least 37 loci based on previous studies on other species (Weeden and Wendel, 1989). Of the 37 loci, 16 loci were polymorphic and P = 43.2% (no criterion; Table 4, Table 5). At the 0.95 level, however, the parameter was only 13.5%, an indication of very weak polymorphism. Averaged across all loci, yellow foxtail as a species had H = 0.042 and A = 1.54. To estimate the degree of population differentiation, genetic diversity was further partitioned into within- and between-population components (Table 6). Loci ACO3, ACO4 and PGD4 were the most variable and PGM1, MDH4 were the least variable. The values of G_{ST} were generally high across loci except for PGM1, ADK1 and MDH4. Overall, yellow foxtail G_{ST} was 0.73. The most frequently observed genotype occurred in 53 accessions from Belgium, Canada, China, Czechoslovakia, Turkey andthe U.S. (AK, IA, IN, KS, ND, MD, MN, PA, WA, WI, and WY). The allelic composition of this most commonly found genotype was: AAT3-A, ACO2-A, ACO3-B, ACO4-B, ADK1-A, ADK2-A, DIA2-A, IDH3-A, MDH4-A, PGD4-A, PGM1-A, PGM2-B, SKD1-A, SKD2-A, TPI2-A, and TPI3-A.

Distinctive patterns in yellow foxtail genetic diversity were observed at various geographic levels. At the continental level, accessions from Eurasia were nearly as diverse as those from North America on the PCA1 axis, suggesting a similar degree of within-region populational differentiation (Figure 1). Nei's genetic identity between North America and Eurasia was 0.99. Populations from Europe, Asia and North America formed three distinctive clusters, largely due to differences in allele frequencies of ACO3-A, ACO3-B, ACO4-A, ACO4-B, PGD4-A and PGD4-B, as indicated by their eigenvector scores (not shown). The accessions from Eurasia had mostly positive PCA2 scores while those from North America had either low or negative PCA2 scores. Statistically, accessions from Eurasia had a slightly higher genetic diversity than North American accessions (Table 4, 5). This was reflected by all four parameters: number of unique alleles, P, A, and H. Setaria glauca accessions from Eurasia had 9 unique alleles: AAT3-B, ACO2-B, IDH3-B, MDH4-B, PGM1-B, SKD1-C, SKD2-C, TPI2-B, TPI-3B

Table 4. Average allelic frequency by regions, calculated as unweighted arithmetic mean of population frequencies (n = number of populations). N. A., North America; S. US, southern US; N. US, northern US

		S. glauca					S. geniculata				
Loci	Allele	Asia	Europe	N. A.	Eurasia	Total	S. US	N. US	US	Eurasia	Total
		n=11	n=16	n=67	n=27	n=94	n=5	n=10	n=15	n=9	n=24
AAT2	A	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000	0.0863	0.0324
	B	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	1.0000	0.9137	0.9676
AAT3	A	1.0000	0.9375	1.0000	0.9630	0.9894	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.0000	0.0625	0.0000	0.0370	0.0106	0.0000	0.0000	0.0000	0.0000	0.0000
ACO2	A	0.9091	1.0000	1.0000	0.9630	0.9894	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.0909	0.0000	0.0000	0.0370	0.0106	0.0000	0.0000	0.0000	0.0000	0.0000
ACO3	A	0.6182	0.8500	0.1218	0.7556	0.2819	0.0000	0.0000	0.0000	0.4444	0.1667
	B	0.3818	0.1500	0.8782	0.2444	0.7181	1.0000	0.2000	0.4667	0.5556	0.5000
	C	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.8000	0.5333	0.0000	0.3333
ACO4	A	0.5909	0.0313	0.1603	0.2593	0.1782	0.0000	0.0000	0.0000	0.2222	0.0833
	B	0.3182	0.9687	0.7821	0.7037	0.7809	1.0000	0.0000	0.3333	0.2222	0.2917
	C	0.0909	0.0000	0.0577	0.0370	0.0410	0.0000	1.0000	0.6667	0.5556	0.6250
ADK1	A	0.9818	1.0000	0.9936	0.9926	0.9952	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.0182	0.0000	0.0064	0.0074	0.0048	0.0000	0.0000	0.0000	0.0000	0.0000
ADK2	A	0.8515	1.0000	0.9936	0.9395	0.9800	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.1485	0.0000	0.0064	0.0605	0.0200	0.0000	0.0000	0.0000	0.0000	0.0000
DIA2	A	0.8636	0.9896	0.9327	0.9383	0.9267	1.0000	0.0000	0.3333	0.3333	0.3333
	B	0.1364	0.0104	0.0673	0.0617	0.0733	0.0000	1.0000	0.6667	0.6667	0.6667
IDH3	A	0.7334	0.9958	0.8814	0.8889	0.9043	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.1273	0.0042	0.0000	0.0543	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000
	C	0.1394	0.0000	0.1186	0.0568	0.0801	0.0000	0.0000	0.0000	0.0000	0.0000
MDH1	A	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000	0.0123	0.0046
	B	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	1.0000	0.9877	0.9954

Table 4. (continued)

Loci	Allele	S. glauca					S. geniculata				
		Asia	Europe	N. A.	Eurasia	Total	S. US	N. US	US	Eurasia	Total
		n=11	n=16	n=67	n=27	n=94	n=5	n=10	n=15	n=9	n=24
MDH2	A	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000	0.0123	0.0046
	B	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	1.0000	0.9877	0.9954
MDH4	A	1.0000	0.9844	1.0000	0.9907	0.9973	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.0000	0.0156	0.0000	0.0093	0.0027	0.0000	0.0000	0.0000	0.0000	0.0000
PGD4	A	0.6909	0.9875	0.8750	0.8667	0.8739	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.3091	0.0125	0.1250	0.1333	0.1261	0.0000	0.0000	0.0000	0.0000	0.0000
PGM1	A	0.9848	0.9979	1.0000	0.9926	0.9979	0.4166	0.1249	0.2221	0.4198	0.2963
	B	0.0152	0.0021	0.0000	0.0074	0.0021	0.5834	0.8751	0.7779	0.5802	0.7038
PGM2	A	0.0000	0.0000	0.0443	0.0000	0.0184	1.0000	1.0000	1.0000	1.0000	1.0000
	B	1.0000	1.0000	0.9557	1.0000	0.9816	0.0000	0.0000	0.0000	0.0000	0.0000
SKD1	A	0.9818	0.9375	0.9968	0.9556	0.9795	0.8000	0.0000	0.2667	0.0000	0.1667
	B	0.0091	0.0625	0.0032	0.0407	0.0194	0.2000	0.4875	0.3917	0.8889	0.5781
	C	0.0091	0.0000	0.0000	0.0037	0.0011	0.0000	0.5125	0.3417	0.1111	0.2552
SKD2	A	0.8909	1.0000	0.9968	0.9556	0.9795	0.8000	0.0000	0.2667	0.0000	0.1667
	B	0.0182	0.0000	0.0032	0.0074	0.0098	0.2000	0.2875	0.2583	0.8889	0.4948
	C	0.0909	0.0000	0.0000	0.0370	0.0106	0.0000	0.7125	0.4750	0.1111	0.3385
TPI2	A	0.8182	1.0000	1.0000	0.9259	0.9787	1.0000	1.0000	1.0000	1.0000	1.0000
	B	0.1818	0.0000	0.0000	0.0741	0.0213	0.0000	0.0000	0.0000	0.0000	0.0000
TPI3	A	0.8182	1.0000	1.0000	0.9259	0.9787	0.6000	0.7900	0.7267	1.0000	0.8292
	B	0.1818	0.0000	0.0000	0.0741	0.0213	0.4000	0.2100	0.2733	0.0000	0.1708

Table 5. Summary statistics of genetic diversity at 37 Loci in S. glauca. Calculations are based on unweighted arithmetic means of population frequencies (n = number of populations)

Parameters	Asia n=11	Europe n=16	North America n=67	Eurasia n=27	Total n=94
No. of unique alleles	10	2	1	9	10
% of Polymorphic Loci (No criterion)	35.1	24.3	27.0	40.5	43.2
% of Polymorphic Loci (0.95 criterion)	27.0	8.1	13.5	21.6	13.5
Mean no. of alleles per locus	1.46	1.24	1.30	1.51	1.54
Mean panmictic heterozygosity	0.092	0.017	0.034	0.053	0.042

Table 6. Partitioning of total genetic diversity (H_T) into within- (H_S) and between-population portions (D_{ST}) for S. glauca; coefficient of population genetic differentiation (G_{ST}) = D_{ST}:H_T

Loci	H_S	H_T	D_{ST}	G_{ST}
AAT3	0.0000	0.0210	0.0210	1.0000
ACO2	0.0000	0.0210	0.0210	1.0000
ACO3	0.0450	0.4050	0.3600	0.8890
ACO4	0.0510	0.3570	0.3060	0.8580
ADK1	0.0070	0.0100	0.0020	0.2240
ADK2	0.0150	0.0390	0.0240	0.6060
DIA2	0.0430	0.1360	0.0930	0.6870
IDH3	0.0370	0.1760	0.1380	0.7880
MDH4	0.0040	0.0050	0.0010	0.2480
PGD4	0.0250	0.2200	0.1950	0.8850
PGM1	0.0040	0.0040	0.0006	0.1430
PGM2	0.0040	0.0360	0.0320	0.8830
SKD1	0.0110	0.0400	0.0290	0.7250
SKD2	0.0110	0.0400	0.0290	0.7310
TPI2	0.0000	0.0420	0.0420	1.0000
TPI3	0.0000	0.0420	0.0420	1.0000
Mean	0.0161	0.0996	0.0835	0.7292
Mean including 21 invariant loci	0.007	0.043	0.036	---

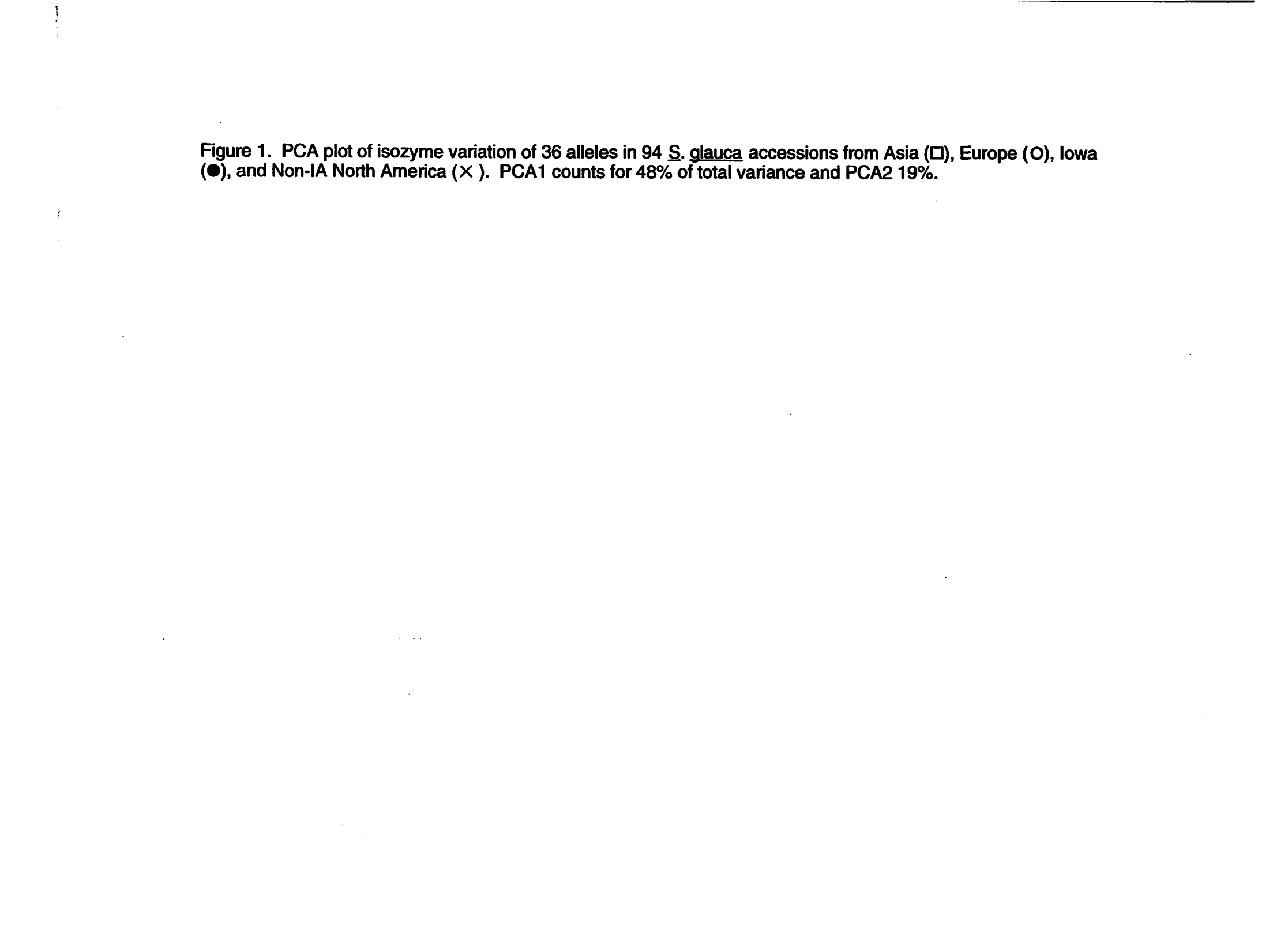
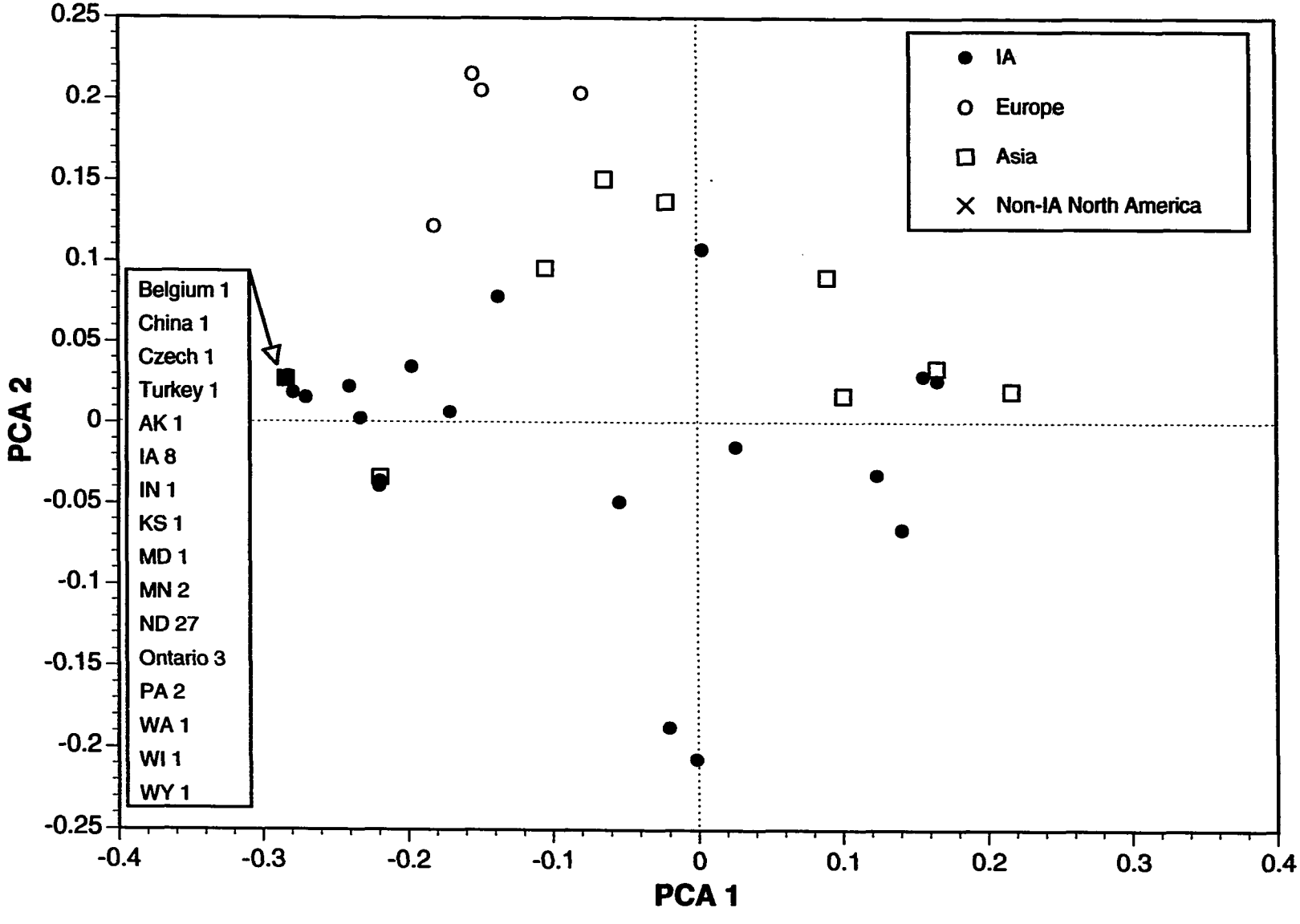


Figure 1. PCA plot of isozyme variation of 36 alleles in 94 S. glauca accessions from Asia (□), Europe (O), Iowa (●), and Non-IA North America (×). PCA1 counts for 48% of total variance and PCA2 19%.


IA
Europe
Asia
Non-IA North America
Belgium 1
China 1
Czech 1
Turkey 1
AK 1
IA 8
IN 1
KS 1
MD 1
MN 2
ND 27
Ontario 3
PA 2
WA 1
WI 1
WY 1
PCA 2
PCA 1
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4

(Table 4). North America accessions had only one unique allele, PGM2-A. Within Eurasia, Asian populations had greater genetic diversity than those from Europe, as indicated by the same statistics (Table 4, 5). Ten alleles were unique to Asian populations: ACO2-B, ACO4-C, ADK1-B, ADK2-B, IDH3-C, SKD1-C, SKD2-B, SKD2-C, TPI-2B, and TPI3-B. Two alleles were unique to European population: AAT3-B and MDH4-B. Within North America, accessions from Iowa were quite diverse whereas those from other states were essentially identical isozymatically (Figure 1). Within state and local regions, populations were either very diverse or homogeneous, as seen in Iowa and Fargo, ND samples. Within the Old World, accessions from Asia formed a distinctive cluster separated from European accessions.

Knotroot foxtail

Genetic diversity in 24 knotroot foxtail accessions was also evaluated using P, A, H and G_{ST} (Table 7). Ten out of 37 loci were polymorphic (no criterion), or P = 27%; at the 0.95 level, P decreased to 19%. For knotroot as a species, A was 1.38, and H was 0.096. To estimate the degree of population differentiation, genetic diversity was further partitioned into within- and between-population components (Table 8). Loci ACO3 and SKD2 were the most variable and MDH1 and MDH2 were the least. Values of G_{ST} were high across all loci except for PGM1. Overall, knotroot foxtail had a G_{ST} of 0.91.

In knotroot foxtail, geographic patterns in genetic diversity were also discernible. Principal component analysis revealed three distinct clusters: Asia (generally 0.15 to 0.25 PCA2, -0.2 to +0.1 PCA1), northern US (generally -0.15 to 0.25 PCA2, 0.2 to 0.4 PCA1), and southern US (generally -0.25 to -0.2 PCA2, -0.3 to -0.2 PCA1) (Figure 2). At the continental level, accessions from Eurasia were less differentiated than those from the U.S. Nei's genetic identity between the two regions was 0.98, indicating little differentiation in allelic content occurred between the Old World and New World. The U.S. group had a slightly greater diversity than did the Eurasian group (Table 7). Although the two groups contained similar numbers of unique alleles, P (0.95 level) and A, the U.S. group had a higher heterozygosity than the Eurasian group. The accessions inthe U.S.

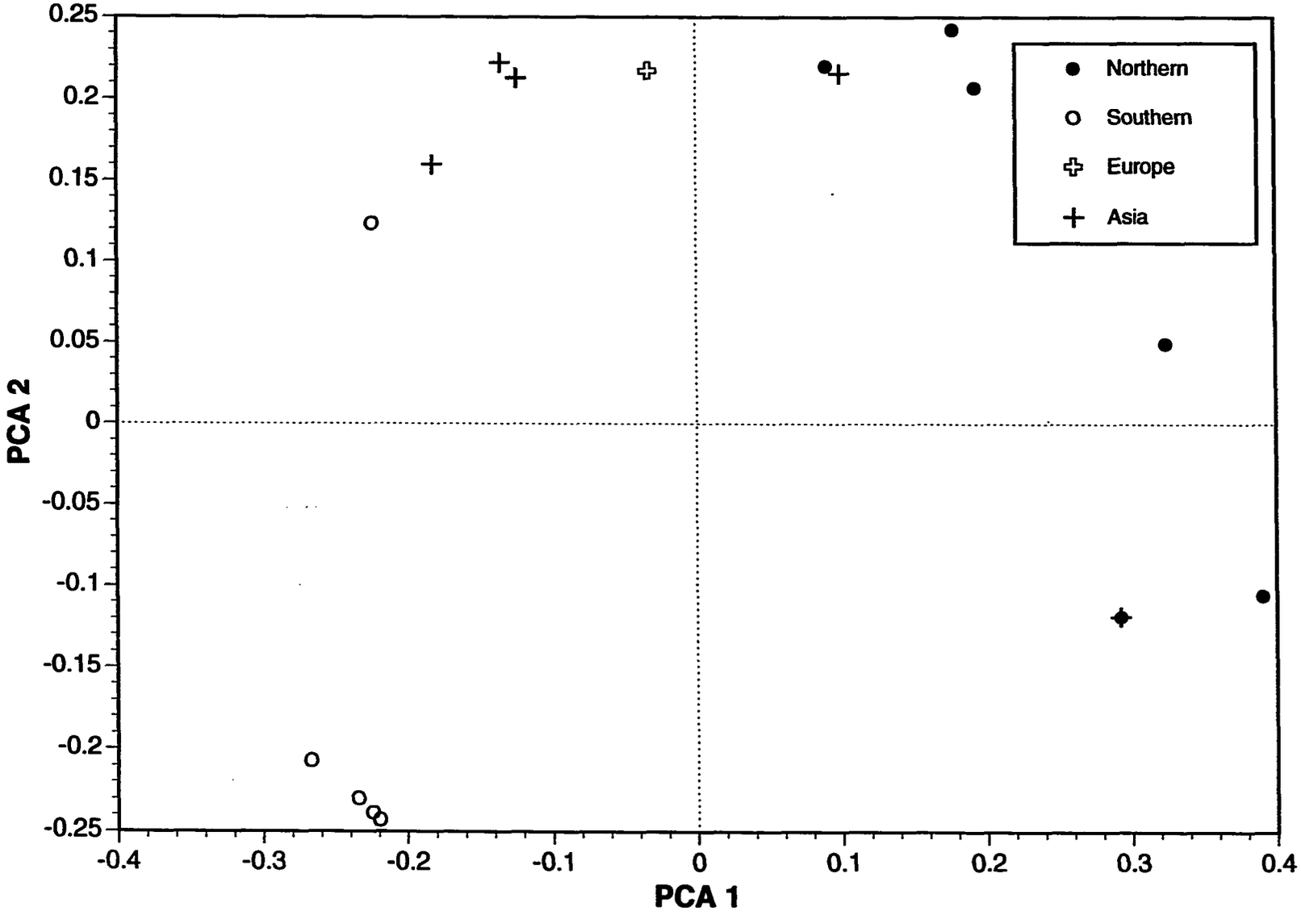
Table 7. Summary statistics of genetic diversity at 37 loci in S. geniculata. S. US, southern US; N. US, northern US

Parameters	S. US n=5	N. US n=10	US n=15	Eurasia n=9	Total n=24
No. of unique alleles	4	5	4	5	4
% of Polymorphic Loci (No criterion)	10.8	13.5	18.9	24.3	27.0
% of Polymorphic Loci (0.95 criterion)	10.8	13.5	18.9	18.9	18.9
Mean no. of alleles per locus	1.11	1.14	1.24	1.27	1.38
Mean panmictic heterozygosity	0.043	0.048	0.093	0.071	0.096

Table 8. Partitioning of total genetic diversity (H_T) into within- (H_S) and between-population portions (D_{ST}) for S. geniculata; coefficient of population genetic differentiation (G_{ST}) = D_{ST}:H_T

Loci	H_S	H_T	D_{ST}	G_{ST}
AAT2	0.0000	0.0627	0.0627	1.0000
ACO3	0.0000	0.6111	0.6111	1.0000
ACO4	0.0000	0.5174	0.5174	1.0000
DIA2	0.0000	0.4444	0.4444	1.0000
MDH1	0.0000	0.0092	0.0092	1.0000
MDH2	0.0000	0.0092	0.0092	1.0000
PGM1	0.2870	0.4170	0.1299	0.3116
SKD1	0.0109	0.5729	0.5619	0.9809
SKD2	0.0109	0.6128	0.6019	0.9822
TPI3	0.0584	0.2833	0.2249	0.7939
Mean	0.0367	0.3540	0.3173	0.9069
Mean including 27 invariant loci	0.0099	0.0957	0.0858	—

Figure 2. PCA plot of isozyme variation of 24 alleles in 24 S. geniculata accessions from the US, Europe and Asia. PCA1 counts for 41% of total variance and PCA2 25%. Northern US (●), Southern US (O), Europe (✣), Asia (+).


0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
PCA 2
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4
PCA 1
Northern
Southern
Europe
Asia

contained four unique alleles: ACO3-C, SKD1-A, SKD2-A, and TPI3-B (Table 4). Five alleles were unique to Eurasia: AAT2-A, ACO3-A, ACO4-A, MDH1-A, and MDH2-A.

Within North America, knotroot foxtail accessions were differentiated into southern and northern groups at about 37°N latitude (the Kansas-Oklahoma border) (Figure 2). Nei's genetic identity between the two groups was 0.90, indicating a greater genetic differentiation within North American populations than between North American and Eurasian populations. Overall, the two North American groups had similar levels of genetic diversity (Table 7). The Northern group contained 5 unique alleles: ACO3-C, ACO4-C, DIA2-B, SKD1-C, and SKD2-C (Table 4). The Southern group included 4 unique alleles: ACO4-B, DIA2-A, SKD1-A, and SKD2-A. No such geographic pattern of genetic diversity was found among Eurasian accessions.

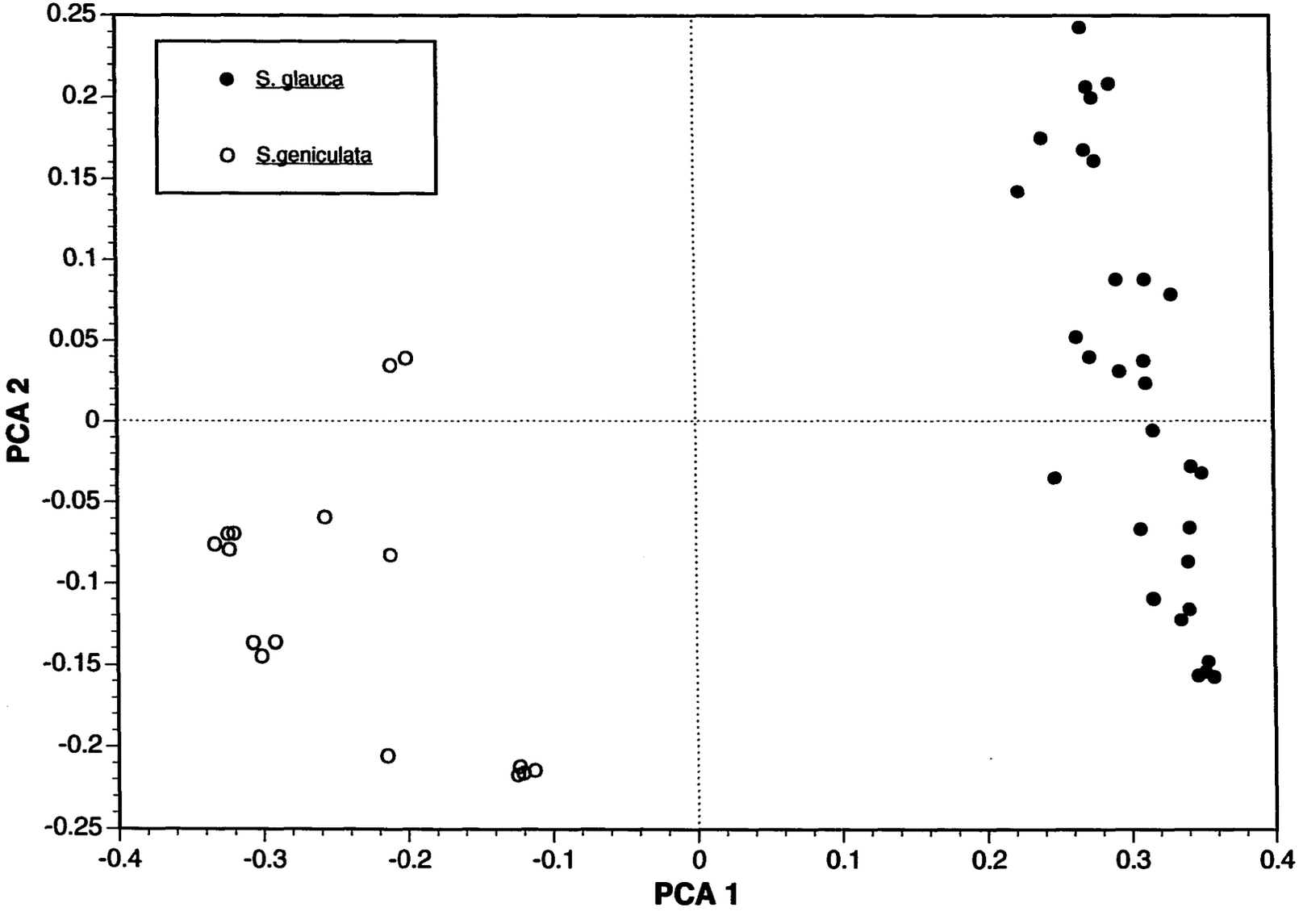
Relationship between yellow and knotroot foxtail

Yellow foxtail is genetically closely related to knotroot foxtail. Nevertheless, populations of the two species formed two distinctive, discontinuous groups with no intermediate populations when analyzed by principal component analysis (Figure 3). Nei's genetic identity between the two species was 0.83. Knotroot foxtail was genetically more diverse than was yellow foxtail. Allelic frequencies were distributed more evenly in knotroot foxtail than in yellow foxtail, leading to greater polymorphism and greater heterozygosity in the former (Table 4, 5, 7). The alleles unique to yellow foxtail were AAT3-B, ACO2-B, ADK1-B, ADK2-B, IDH3-B, IDH3-C, MDH4-B, PGD4-B, PGM2-B, and TPI2-B (Table 4). The alleles unique to knotroot foxtail were AAT2-B, ACO3-C, MDH1-B, MDH2-B. In addition, yellow foxtail had high frequencies of AAT2-A, ACO4-B, DIA2-A, MDH1-A, MDH2-A, PGM1-A, SKD1-A, and SKD2-A. Knotroot foxtail had high frequencies of ACO4-C, DIA2-B, PGM1-B, PGM2-A, SKD1-B, SKD1-C, SKD2-B, SKD2-C, and TPI3-B.

Giant foxtail

A survey of 51 accessions of giant foxtail revealed very little isozyme polymorphism. One population, from Vladivostock, Russia (accession 2448)

Figure 3. PCA plot of isozyme variation of 36 alleles in 94 S. glauca (●) and 24 S. geniculata (O) accessions. PCA1 counts for 65% of total variance and PCA2 11%.


S. glauca
S.geniculata
PCA 2
PCA 1
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4

had two polymorphic loci, PGI and PGD, with two alleles each. Otherwise, giant foxtail was isozymatically invariant.

Discussion

Genetic diversity, structure and weedy adaptation

Insights into foxtail's evolutinary history can be made from analyzing foxtail's genetic diversity and population genetic structure, both of which are the outcomes of the complex interaction, over time, between environment (natural selection), genetics (mutation, random genetic drift and gene flow), and dispersal. Equally important is the genetic relationship between yellow and knotroot foxtail.

Genetic diversity Yellow foxtail and knotroot foxtail contained little genetic diversity within the species, but had marked differentiation between populations, compared to other plant species. An extensive review of genetic diversity in many plant species has been made (Hamrick and Godt, 1990). An average plant species had a P = 50% (criterion unknown), A = 1.96 and H of 0.15 at the species level. At the population level, P, A and H were 34%, 1.53 and 0.11 respectively. The average G_{ST} was 0.22. In contrast, yellow foxtail as a species had P = 13.5% (0.95 criterion), A = 1.54 and H = 0.042 (Table 5). For knotroot foxtail, P, A and H were 18.9%, 1.38 and 0.096 respectively (Table 7). Therefore, both species exhibited genetic diversities below the average of other plant species. When the total genetic diversity was further partitioned into within- and between population components, yellow and knotroot foxtail showed much stronger population differentiation than the average plant species, with G_{ST} = 0.73 and 0.91, respectively (Table 6, 8).

Some isozyme studies of genetic diversity in weed species have been reported (summarized in Wang et al., 1994). The genetic diversity in knotroot and yellow foxtail were generally comparable to that of other self-pollinating weedy species reported in previous literature. For example, Sorghum halepense had a P = 14% (criterion unknown). Echinochloa oryzoides had a P = 16% (criterion unknown), A = 1.16 and G_{ST} = 0.50. Pyrrhopappus carolinianus had a P = 25% (0.99 criterion), A = 1.29, H = 0.07 and G_{ST} =

0.47 (Petersen et al., 1990). Overall, yellow and knotroot foxtail had the low genetic diversity with strong population differentiation resembling other typical self-pollinating weeds (Barrett, 1988; Barrett and Shore, 1989; Barrett and Richardson, 1986).

Patterns of genetic diversity There were geographic patterns in the genetic diversity of yellow and knotroot foxtail. Yellow foxtail contained greater genetic diversity in its native range (Eurasia) than it did in its introduced range (North America) (Table 5). Yellow foxtail populations in Eurasia also had more unique alleles than those in North America. Yellow foxtail from Asia, Europe and North America formed three distinctive clusters. Despite these differences between the Old and New World, genetic divergence between yellow foxtails of the two continents appeared to be minimal, as indicated by the Nei's genetic identity of 0.99. In North America, the accessions from Iowa were the most diverse, whereas those from other locations in the continent, including 27 from Fargo area, ND, were essentially identical (Figure 1). This most frequently observed genotype occurred in 53 accessions from 6 countries, in both the Old World and the New World. Its allelic composition was: AAT3-A, ACO2-A, ACO3-B, ACO4-B, ADK1-A, ADK2-A, DIA2-A, IDH3-A, MDH4-A, PGD4-A, PGM1-A, PGM2-B, SKD1-A, SKD2-A, TPI2-A, TPI3-A. All 16 loci were fixed in these 53 accessions.

In knotroot foxtail, greater genetic diversity occurred in the native range (the U.S.) than that found in the introduced range (Eurasia; Table 7). Genetic divergence between knotroot foxtail from the two continents appeared to be minimal, as indicated by the Nei's genetic identity of 0.98. Accessions from Eurasia were less differentiated inter se than were those from the U.S. (Figure 2). North-south regional differentiation occurred among the U.S. accessions along a line at about 37°N latitude (the Kansas-Oklahoma border; Figure 2). Nei's genetic identity between the northern and southern group was 0.90, indicating a greater genetic differentiation within North American knotroot foxtail populations than between North American and Eurasian populations. A similarly strong intra-continental differentiation was also observed in green foxtail populations, both in Eurasia and North America (Jusuf and Pernes, 1985; Wang et al., 1994).

For both yellow and knotroot foxtail, there were interregional differences in genetic diversity between Eurasia and North American populations. Populations from the native range were more diverse than those from the introduced range, as measured by parameters describing both allelic richness and allelic evenness (Table 5, 7). This was expected because presumably migration of these species involved a partial sampling of original total gene pool or founder effect. On the other hand, the difference in genetic diversity between the populations in Eurasia and North America was rather small, and the genetic divergence between the two continents was minimal. Introduction probably occurred more than once in the past, from Eurasia to North America for yellow foxtail and in the opposite direction for knotroot foxtail.

Geographic patterns of genetic variability were observed for both species. Yellow foxtail diverged into European, Asian and North American clusters. The Asian cluster was genetically the most diverse among the three even though it had the smallest number of samples (Table 5). This finding seems to suggest that yellow foxtail was originated in Asia, and that yellow foxtail in North America was introduced largely from Asia rather than Europe. The pattern of genetic variability of yellow foxtail populations in North America was more difficult to interpret. Why were Iowan accessions so diverse whereas those from elsewhere in the continent homogeneous? The answer to this question is not apparent. It may be a function of historical introduction events: one or few introductions for most regions of the U.S., associated with founder effects, but multiple introductions to Iowa leading to greater diversity. Alternatively, the sampling of yellow foxtail could be somehow seriously biased, producing this skewed pattern. Distinctive geographic patterns in yellow foxtail may also be explained by its intraspecific differences in ploidy levels, which could cause reproductive isolation among some populations thus leading to population differentiation. The north-south regional differentiation of knotroot foxtail in North America was probably a result of natural selection and local adaptation. Knotroot foxtail is known to favor the warmer and moister southern conditions, relative to those in the northern U.S. (Rominger, 1962).

Weedy adaptation The population genetic structure and evolutionary success of self-pollinating weed colonizers strongly suggest that abundant genetic variation is not a prerequisite for being a successful colonizer (Barrett and Shore, 1989). Two adaptive strategies were hypothesized to be the reasons these colonizers are so successful: genetic polymorphism with locally adapted genotypes ("specialists"), and phenotypic plasticity with "general purpose" genotypes ("generalists") (Baker, 1965, 1974; Bradshaw, 1965; Barrett and Richardson, 1986).

Yellow and knotroot foxtails were low in genetic diversity but had marked population differentiation. For instance, in yellow foxtail, relatively strong genetic differentiation occurred to populations in Iowa. On the other hand, populations from widely separated ecological and geographic locations may be identical. This is the case with a common yellow foxtail genotype. It was found in 53 accessions distributed in 6 countries from Europe, Asia and North America: the soybean fields of northern Manchuria, China; Turkey; the lowlands of Belgium in northern Europe; along the Labe (Elbe) River valley of northern Bohemia in Czechoslovakia; the agricultural highlands of southern Ontario, Canada; and from disturbed habitats (e. g., agricultural fields, waste areas, roadsides) in all the U.S. states (east: MD, PA; west: WA, WY; south: AK; north and midwest: IA, IN, KS, MN, ND, WI) sampled. The adaptive significance, if any, of these genetically divergent versus common genotypes remains to be revealed.

Relationship between S. glauca and S. geniculata

The relationship between S. glauca and S. geniculata is an enigma. They supposedly originated in two different continents, yet they are morphologically and genetically quite similar (Rominger, 1962; Chikara and Gupta, 1980). In fact, all of the twenty-four accessions of knotroot foxtail in this study were initially identified as yellow foxtail based on their morphology. Isozymatically the two species formed two distinctive, discontinuous groups in the PCA plot (Figure 3). Despite this, their genetic identity was 0.83, a relatively high estimate for two different species (reviewed in Pleasants and Wendel, 1989). Genetic diversity was greater in knotroot foxtail than in yellow foxtail. Knotroot foxtail resembles some foxtail species in tropical

Africa (Rominger, 1962). Given that the African continent might be the center of origin of the genus Setaria, it is not unlikely that these two species share a common African ancestor from their evolutionary past.

The weedy foxtail species-group

Several conclusions can be drawn regarding the genetic diversity and population genetic structure in weedy foxtails. Most of the weedy foxtail species studied have lower genetic diversity, but higher population differentiation, than does the average plant species (Hamrick and Godt, 1990). This low diversity and high population differentiation in the foxtails was similar to that found in other introduced, self-pollinating weeds (Barrett, 1988; Barrett and Richardson, 1986; Barrett and Shore, 1989; Brown and Marshall, 1981; Rice and Jain, 1985).

Within the foxtail species-group, knotroot foxtail had the highest diversity, followed by green, yellow and giant foxtail (Table 9). Knotroot foxtail populations were also the most strongly differentiated genetically, followed by yellow, green and giant foxtail. The implication of these differences in foxtail weedy adaptation was not clear and many questions remained unanswered. Geographic patterns of green, yellow and knotroot foxtail genotypes may indicate adaptation to those different ecological situations by specialized genotypes. Conversely, generally adapted genotypes of green, yellow and giant foxtail may indicate that a common population is capable of broad adaptation through enhanced phenotypic plasticity. These important questions of adaptive strategies utilized amongst foxtail genotypes await further studies with the populations identified in these reports.

Geographic patterns in genetic diversity were observed in both green, yellow and knotroot foxtails, as well as in many other plant species (Barrett and Husband, 1990; Bergmann, 1978; Bretting et al., 1990; Kahler and Price, 1986; Lundkvist and Rudin, 1977; Nevo et al., 1979; Wendel and Percy, 1990; Wendel and Parks, 1985; Yang et al., 1977; Yeh and O'Malley, 1980). Presumably, populations in these different ecogeographic regions were subjected to differential natural selection. Genotypes adapted to local conditions were favored and became more prevalent in that environment.

Yellow foxtail populations formed three distinct world clusters: Asia, Europe and North America. The greater diversity in the Asian populations may be an

Table 9. A summary of the genetic diversity and population genetic structure of weedy foxtails by isozyme studies

Species	Relative Rank in Genetic Diversity	Relative Rank in Population Differentiation	Geographic Patterns in Genetic Diversity
S. geniculata	high	high	northern and southern clusters in North America
S. glauca	low	medium	Asian, European and North American clusters; diverse Iowan populations vs. other identical populations from the rest of North America
S. viridis	medium	low	northern and southern clusters in North America; diverse Iowan populations
S. faberii	very low	very low	no

indication of yellow foxtail's origins in that area. A striking lack of diversity amongst non-Iowa yellow foxtail populations was observed, and may indicate either generalized adaptation to diverse ecological conditions or restricted diversity from limited historical introductions into the U.S.

North-south regional differentiation in North America was observed in knotroot and green foxtail. It was perhaps not a coincidence that both knotroot and green foxtails differentiated along a north-south gradient. It has been generally observed that genetic diversity of biological organisms changes along the north-south axis, with genetic diversity increasing as the latitude decreases (Stevens, 1989). The difference in the dividing line (knotroot, 37° N; green, 43.5° N) in North America between these two species may be a function of the environments favoring their growth. Knotroot foxtail favors the warm, moist southern U.S. whereas green foxtail tends to thrive in the northern parts of this continent (Rominger, 1962).

Both green and yellow foxtail populations in Iowa had relatively high genetic diversity compared to populations from the rest of North America (Wang et al., 1994). This unusual pattern may be largely the result of the historical events during the introduction of foxtails from Eurasia to North America. There were probably multiple introductions of green and yellow foxtail to Iowa. Alternatively, diverse foxtail populations may be maintained by environmental heterogeneity in Iowa, which is in the center of the north-south gradient in North America and

genetically divergent populations may coexist there. A third possibility is that foxtails in Iowa were relatively oversampled and overrepresented.

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WEEDY ADAPTATION IN SETARIA SPP.: III. VARIATION IN HERBICIDE RESISTANCE IN SETARIA SPP.

A paper to be submitted to the Journal of Pesticide Biochemistry and Physiology

Rong Lin Wang and Jack Dekker

Abstract: Setaria is a grass genus of about 125 species that includes both food crops and a number of important agricultural weeds. Setaria viridis, S. faberii, S. glauca, and S. geniculata are major agricultural weeds worldwide and in North America. There is currently an inadequate knowledge of inter- and intra-specific herbicide resistance variation in these foxtail species, in spite of the importance of this knowledge to understanding evolution of herbicide resistance and improving weed management. Previous isozyme analyses of these species indicate that significant variation in genetic diversity exists among foxtail populations. It is unknown whether this genetic diversity is correlated with variability in important adaptive traits such as herbicide resistance. Studies were conducted to determine if inter- and intra-specific differences in atrazine and metolachlor resistance exist in foxtail species. Three assays were utilized to make these determinations: whole plant dose-response, in vivo leaf chlorphyll fluorescence, and glutathione S-transferase conjugation (GST) assays. Significant variations in atrazine and metolachlor resistance were revealed within and among foxtail species. Green and giant foxtail were more resistant to atrazine than was yellow foxtail. Although green and giant foxtail again had a similar level of resistance, yellow foxtail was the most resistant species to metolachlor. These results indicated that the resistance mechanisms (quantitative or qualitative) to these two herbicides may be different in yellow, green and giant foxtail. Intra-specific differences in atrazine resistance were found within both green foxtail populations, and with yellow foxtail populations. Intra-specific metolachlor resistance differences were detected amongst green foxtail populations, but not in other foxtail species. No evidence for population shifts to more resistant foxtail variants with prolonged atrazine exposure was found in several detailed studies. When populations from several farms with a long history of atrazine use were compared, no differences in atrazine

resistance were detected among populations from treated areas and adjacent untreated areas. Chlorophyll fluorescence assays indicated a similar pattern of atrazine resistance among foxtail populations, although it was less sensitive in detecting differences than the whole plant assay. No differences in GST-mediated atrazine or metolachlor conjugation were detected within or between foxtail species. These results may indicate that GST-mediated glutathione-herbicide conjugation is not the primary detoxification mechanism for these herbicides in these foxtail species. Several foxtail species had significant inter- and intraspecific differences in GST-mediated CDNB conjugation activity. In some instances these responses were similar to those observed in the whole plant responses to metolachlor, although the significance of these similarities was not clear.

Introduction

The weedy foxtails (Setaria spp.) are successful colonizing weeds of agroecosystems due to their worldwide distribution, great biological diversity and competitiveness in disturbed habitats (Hafliger and Scholz,1980; Lorenzi and Jeffery, 1987; Wang et al., 1994a, b). They are important agricultural weeds worldwide and major weeds in the United States (Holm et al., 1977; Anonymous, 1987). Despite their importance, much of their biology is poorly understood. For example, little is known of the population biology determining their geographic distribution and competitive ability. Lack of such knowledge has seriously hindered the progress of developing new weed management strategies. Previous studies of green (Setaria viridis), yellow (S. glauca), knotroot (S. geniculata), and giant foxtail (S. faberii) allozyme diversity revealed considerable variability between and amongst these species, as well as strongly differentiated populations (Wang et al., 1994a, b).

Populations in a given geographic range could be either genetically well-differentiated or homogeneous. Since a large amount of genetic diversity is an unlikely requirement for being a successful weed, it is possible that a combination of existing levels of genetic diversity, phenotypic plasticity and spatial heterogeneity will result in variation in adaptive traits, such as herbicide resistance. As significant endemic weeds of agroecosystems, the foxtails have

been exposed to strong selection pressure from herbicides. This long term exposure has been responsible for development of altered binding site resistance (D-1 protein) in several of the foxtail species (Thornhill and Dekker 1993, Bandeen et al., 1982; De Prado et al., 1989; Warwick, 1991). To date, no herbicide resistant foxtail mutants due to herbicide metabolism have been reported. There remains the possibility that incremental increases in metabolic detoxification due to herbicide selection has occurred in populations with long histories of herbicide exposure. What remains unknown is the diversity of metabolic herbicide resistance phenotypes within and between the weedy foxtail species, within which selection could occur.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) and metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide) are two important herbicides used to control the foxtails (Hartzler and Owen, 1993). Previous studies have indicated a relative order of resistance to atrazine (greatest to least): S. viridis > S. faberii = S. glauca (Thompson, 1972; Jensen, 1977). Foxtail resistance to metoloachlor appears to be dependent on the rate applied: at high dosages (greater than 1.1 kg/ha) all species were susceptible; at low dosages, it appeared that yellow foxtail was more resistant than the other species. Intraspecific resistance has been observed in two of the foxtail species: dalapon (sodium 2,2-dichloropropionate) resistance among yellow foxtail populations; and s-triazine resistance among green foxtail populations (Santeimann and Meade, 1961; Schreiber and Oliver, 1971).

Chlorphyll fluorescence has been widely used in probing plant stress responses and herbicide detoxification (reviewed in Bolhar-Nordenkampf, 1989; Papageorgiou, 1975; Schreiber, 1983; Voss et al, 1984; Ducruet et al, 1984; Richard et al, 1983; Dekker and Burmester, 1988; Bohme et al, 1981; Pfister and Arntzen, 1979; Renger, 1986; Cadahia et al, 1982). It is a rapid, non-destructive, intrinsic probe of photosystem II electron transport in the chloroplast. It is used to assess inhibition and damage to photosynthetic electron transfer, the primary site of action of atrazine. Chlorophyll fluorescence measures the "Kautsky effect", a phenomenon characterized by several measurable parameters (Kautsky, 1931). Changes in variable

fluorescence over the period of herbicide treatment, inhibition and recovery to pretreatment levels reflect the action and detoxification of the inhibitor.

One of the most important detoxification mechanisms for both atrazine and metolachlor in many species is herbicide conjugation to glutathione mediated by glutathione S-transferase (GST). This enzyme is important in the metabolism of many other herbicides, and is also important in biological organisms for detoxifying other kinds of xenobiotics (Lamoureux and Frear, 1979). It has multiple isozymes with activities against a broad range of substrates which have electrophilic groups. The activity against one such substrate, CDNB (1-chloro-2,4-dinitrobenzene), is often accepted as a measure of its general enzymatic activity. In plant species, there is ample evidence that GST is involved in atrazine and metolachlor metabolism with either constitutive or inducible activities (Hatzios and Penner, 1982; Dean et al., 1990; Gronwald et al., 1987, 1989). In Setaria GST conjugation is more important than dealkylation and hydroxylation, but its total activity is much less than that in resistant species such as maize (Zea mays) (Jensen et al., 1977).

Inter- and intraspecific variation in herbicide resistance amongst the weedy foxtails may have important implications in terms of the evolution and spread of resistant biotypes, weed management, population genetic structure and could provide an important step toward understanding foxtail weedy adaptation. Based on the earlier studies in this series (Wang et al., 1994a, b), we hypothesize that there are both inter- and intraspecific variations in atrazine and metolachlor metabolic resistance in several foxtail species.

Materials and Methods

A dosage response assay was utilized to assess the variation in herbicide resistance amongst foxtail populations at the whole plant level. This variation was then assessed with a chlorophll fluorescence emission assay in vivo, and GST assays in vitro, in an attempt to explain the physiological basis of variation in herbicide metabolism found at the whole plant level.

Plant material

Four Setaria species were evaluated: green, yellow, giant and knotroot foxtails (Table 1). Some of the populations utilized were those previously

Table 1. List of Setaria spp. accessions used for inter- and intraspecific comparisons; DR, dose response assay; CF, chlorophyll fluorescence assay; GST, glutathione S-transferase assay

Species	Accession	Location	Ecological sites	Assays used
INTER- AND INTRASPECIFIC COMPARISONS				
S. faberii	25	Woodstock, IL		DR, CF, GST
S. geniculata	1487	MD		CF
S. geniculata	1544	OH		CF
S. geniculata	1548	KS		CF
S. geniculata	1747	Harrisburg, AK		CF
S. geniculata	1751	Clarkdale, AK		CF
S. glauca	98	Ames, IA	farm field	DR, CF, GST
S. glauca	901	Lansing, IA	farm field	DR, CF, GST
S. glauca	1037	Johnson Co., IA	roadside	DR, CF, GST
S. viridis	110	Ames, IA	farm field	CF
S. viridis	893	Decorah, IA	farm field	CF
S. viridis	895	Decorah, IA	farm field	CF
S. viridis	902	Marble Rock, IA	roadside	CF
S. viridis	1144	Keokuk Co., IA	roadside	DR, CF, GST
S. viridis	1219	Allision, IA	roadside	DR, CF, GST
S. viridis	1266	Luther, IA	farm field	CF
S. viridis	1273	Beltsville, MD		DR
S. viridis	1278	Madision, WI		DR, CF, GST
S. viridis	1322	Hampton, IA	farm field	DR, CF, GST
S. viridis	1763	Wyndmere, ND	farm field	CF
S. viridis	1801	Embden, ND	farm field	CF
AMONG TRANSECT COMPARISONS AT IOWA FARMS				
S. faberii	840	Hampton, IA	tilled field, 1m from fence	CF
S. faberii	841	Hampton, IA	untilled fence row	CF
S. faberii	842	Hampton, IA	bulk collection from farm field	CF
S. faberii	881	Lansing, IA	untilled fence row	CF
S. faberii	884	Lansing, IA	tilled field, 1m from fence	CF
S. viridis	655	Holy Cross, IA	tilled field, 9m from fence	CF
S. viridis	656	Holy Cross, IA	untilled fence row	CF
S. viridis	657	Holy Cross, IA	tilled field, 1m from fence	CF
S. viridis	660	Holy Cross, IA	roadside	CF
S. viridis	729	Washington, IA	tilled field, 1m from fence	CF
S. viridis	730	Washington, IA	tilled field, 1m from fence	CF
S. viridis	732	Washington, IA	roadside	CF
S. viridis	895	Decorah, IA	bulk collection from ditches adjacent to farm field	CF
S. viridis	896	Decorah, IA	untilled fence row	CF
S. viridis	898	Decorah, IA	tilled field, 7m from fence	CF

analyzed with isozyme markers, and were those representing the maximum genetic differentiation within species (Wang et al., 1994a, b). Other populations came from several Iowa farms with long-term histories of atrazine use (ca. 20 years; Thornhill and Dekker, 1993). Seeds were collected from several 30 m by 1 m transects, from the untilled fence row at the edge of the field to 80 m inside the tilled field. The distance between transects varied from about 1 m to 20 m. Seeds were germinated and grown in a greenhouse with 25°C temperature and 16 h light:8 h dark photoperiod. The soil mix was 1 vermiculite:1 soil:1 peat moss. In the dosage response assay, seeds were planted in 5.4 by 5.4 by 5.4 cm cells, treated with herbicides and left to germinate in greenhouse. In the chlorophyll fluorescence assay, plants were transplanted to 12 by 12 by 12 cm pots at the 3 leaf stage. Three days after transplanting, they were moved to controlled environment growth chambers: constant 25°C temperature; 16 h light:8 h dark photoperiod; and light intensity was about 800 $\mu M\ m^{-2}\ s^{-1}$. In the GST assay, plants established in the greenhouse were harvested at the 2.5-3.0 leaf stage, when the leaves were about 10 cm long.

Dose response assay

The purpose of this experiment is to study the variation in herbicide resistance at the whole plant level, by measuring the reduction of seedling fresh weight after the seeds were initially treated with herbicides at various dosage during planting. The rates for atrazine were 0, 0.28, 0.56, 1.11, and 2.23 kg $hectare^{-1}$ active ingredient (a. i.).; for metolachlor they were 0, 0.017, 0.035, 0.069 and 0.139 kg $hectare^{-1}$ (a. i). Seeds from selected populations were planted in small cells in a flat so that there would be approximately 50 emerged plants per cell. The experiment was designed as a randomized complete block with three replicates (three flats). Each replicate consisted of three subsamples (three cells). Flats were treated in a closed chamber with a motor-driven spray jet. During a spray, the jet moved at a constant speed of 22 cm sec^{-1} with the system air pressure maintained at 2.8 kg cm^{-2}. The output volume was 2.64 μl cm^{-2}. Each cell had a surface area of 29 cm^{-2}, and received a total of 76 μl herbicide at a given concentration. The coefficient of variation in output volume between sprays was about 6%. About 10 days after the treatment, the three leaf

stage plant shoots from each cell were harvested, counted and weighed. The average fresh weight per plant was calculated and converted to a percentage of the untreated control. Regression analysis was then performed on this data and line equations of herbicide dose versus plant weight generated. Analysis of variance and Duncan's multiple range test were then performed on the slopes from these equations to compare the responses of the several foxtail populations.

Chlorophyll fluorescence assay

The purpose of this experiment was to learn whether the variation in atrazine resistance at the whole plant level could be explained by atrazine detoxification over time in leaf tissues as revealed by chlorophyll fluorescence (LCF). Three to four plants were selected from each population when they reached the 3-4 leaf stage with 2-3 basal tillers. An area of about 0.5 cm^2 on the topmost, fully-expanded leaf was marked out by two strips (1 mm wide) of transparent Scotch tape. Fluorescence measurements were taken on this same area of the leaf at different times over a period of 24 h with a chlorophyll fluorometer (model SF-20, Richard Brankner Research Ltd., Ottawa, Canada). Each measurement was preceded by a 10 min. dark adaptation period. After the first (untreated) measurement, a 5 µl atrazine droplet ($1x10^{-4}$ M atrazine; 0.5% crop oil concentrate, a mixture of emulsifier and petroleum based oils; and 0.5% methanol) was applied to the marked spots. Between measurements, the plants were kept illuminated (about 800 µM m^{-2} s^{-1}). After completion of the experiment, the fluorescence data was analyzed and several indices were calculated: F_o (fluorescence at the onset of light after dark adaptation), F_i (fluorescence at the inflection point), and $t(F_i)$ (time from the onset until the inflection point) of pretreatment (untreated) curves for each population; and the corresponding treatment F_i values at the $t(F_i)$ calculated from the pretreatment (untreated) fluorescence induction curves. The differences between the pretreatment F_i value and all the subsequent treatment F_i values were calculated. The initial difference (the maximum inhibition) was taken as one hundred percent and all the later differences were standardized accordingly. The decline of these percentage values over time was plotted. The line equation of this function was obtained by means of

regression analysis. The equation slope represents the detoxification rate of that foxtail population. Analysis of variance and Duncan's multiple range tests were then performed on the slopes from these equations to compare the responses of the several foxtail populations. Due to the critical influence of environmental conditions on foxtail plant tissues in these LCF assays, and how they interact to influence atrazine detoxification kinetics, statements of differences between populations were only reliable when made within the same group of populations or the same experiment.

Glutathione S-transferase assays

Experiments were performed to evaluate the role GST conjugation might play in the detoxification of the two herbicides in different foxtail populations. The procedure used was based on that of Anderson and Gronwald (1991), Gronwald et al. (1987), Habig and Jakoby (1981), and Lamoureux and Rusness (personal communications, USDA-ARS, Fargo, ND) with some modifications. The experiments were conducted using a completely randomized design with three replicates (three plants for each foxtail population). Three substrates were assayed: CDNB (artificial substrate), ^{14}C-atrazine and ^{14}C-metolachlor. Rates of nonenzymatic conjugation for all three substrates were subtracted from the measurements when enzyme activities were calculated. Analysis of variance and Duncan's multiple range tests on population means were then performed.

Enzyme preparation Four grams of leaf tissue per plant was ground in a cold mortar in liquid N_2 and the tissue powder stored at -80° for no more than 21 days. Tests indicated such storage had no adverse effect on enzyme activity (data not shown). The tissue extraction buffer consisted of 20.0 ml ice-cold 0.1 M potassium phosphate solution at pH 6.8, 20 mM mercaptoethanol, 2 mM EDTA (sodium salt of ethylenediaminetetraacetic acid), 10 mM GSH (reduced glutathione), 5% insoluble PVPP (polyvinylpolypyrrolidone), 2.5% BSA (bovine serum albumin), 1 mM PMSF (phenyimethylsulfonyl fluoride) and some acid-washed sand. The buffer to tissue ratio was 5 ml gram^{-1} tissue. The homogenate was filtered and centrifuged at 4°C at 20,000 g for 20 min. The supernatant was collected and fractionated with ammonium sulfate at various saturation ranges. The pH was adjusted to about 6.5 with 1 N NH_4OH solution.

Ammonium sulfate precipitation did not change GST activities (data not shown). Proteins precipitated in the 40%-60% saturation fraction were redissolved and desalted using a PD-10 Sephadex G-25 column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). This desalted fraction was the enzyme extract used in later assays. Based on the CDNB activity, the enzyme was purified about 4.7-fold and the recovery was 125%. The higher than 100% recovery was due to the removal of some small endogenous inhibitors during purification, a commonly observed phenomenon in GST studies (Frear and Swanson, 1970; Lamoureux, Rustness and Gronwald, personal communications).

GST-CDNB activity GST catalyzes the conjugation reaction between GSH and CDNB, an artificial substrate (Habig and Jakoby, 1981). The reaction was monitored spectrophotometrically with time. The reaction rates were calculated from the changes in absorbance over time using the molar extinction coefficient of 9.6 O.D. mM^{-1} cm^{-1}. First, 0.01 ml enzyme extract (about 0.3 mg protein), 1.99 ml of 0.1 M potassium phosphate buffer at pH 6.5, and 0.9 ml of 33 mM GSH were mixed together and incubated in a water bath for 10min. at 25°C. Then, the reaction was started by the addition of 0.1 ml of 48 mM CDNB (dissolved in absolute ethanol) to the preincubated mixture described above. The change in absorbance at 340 nm over the first 5 min. was monitored spectrophotometrically at 25°C with readings taken every 30 sec.

GST -^{14}C-metolachlor activity GST catalyzes the conjugation between GSH and metolachlor, thus rendering the herbicide inactive. The reaction rate of this conjugation was determined by recording the amount of ^{14}C-metolachlor incorporated into GS-metolachlor conjugate per unit of time. Two separate mediums were preincubated simultaneously for 15 min. at 30°C. The first medium was a mixture of 0.1 ml of 120 mM GSH; 0.79 ml of 0.1 M potassium phosphate buffer at pH 7.6, and 0.15 ml of enzyme extract (approximately 4.5 mg protein). The second medium was 0.16 ml 15.1 mM ^{14}C-metolachlor (specific activity 0.314 cpm $pmole^{-1}$) in 31% dimethylformamide. The final concentration of ^{14}C-metolachlor in the reaction mixture was 2.01 mM with a total of about 758,000 cpm. The reaction was started by mixing the two preincubated mediums together at 30°C. Aliquots of 150 µl of the reaction

mixture were taken out every 6 min for 30 min and put into Eppendorf tubes (0.5 ml) containing 150 µl methyiene chloride to stop the reaction. The final concentration of dimethylformamide in the reaction mixture was 4.1%. Tests indicated that 4.1% dimethylformamide had no adverse effect on enzyme activity (data not presented).

GST -^{14}C-atrazine activity GST can also catalyze the conjugation between GSH and atrazine to render the herbicide inactive. The reaction rate of this conjugation was measured by recording the amount of ^{14}C-atrazine incorporated into GS-atrazine conjugate per unit of time. Two separate mediums were preincubated for 15 min at 30°C. One contained 0.1 ml of 120 mM GSH, 0.75 ml of 0.1M potassium phosphate buffer at pH 6.8, and 0.15 ml of enzyme extract (approximately 0.49 mg protein). The second medium has 0.20 ml of 3.703 mM ^{14}C-atrazine (specific activity1.53 cpm pmoie^{-1}) in 25% ethanol. The final concentration of ^{14}C-atrazine in the reaction mixture was 0.617 mM. The total counts per assay was about 1,133,000 cpm. The reaction was started by mixing the two preincubated mediums together at 30°C. Aliquots of 150 µl reaction mixture were taken out every 6 min for 30 min and put into Eppendorf tubes (0.5 ml) containing 150 µl methylene chloride to stop reaction. The final concentration of ethanol in the reaction mixture was 4.1%. Tests indicated that 4.1% ethanol had no adverse effects on the enzyme activity (data not presented).

Results

Dose response assay

Inter-specific variation in herbicide resistance Green and giant foxtails were inhibited similarly by atrazine, and both were more resistant to the herbicide than yellow foxtail (Figure 1). A given dosage of atrazine was about two times more effective inhibiting yellow foxtail than it was for giant or green foxtail. No rate tested completely inhibited growth of any of the species. Atrazine rates above 0.50 kg/ha (a. i.) resulted in only small increases in growth inhibition of all three species.

Green and giant foxtails were inhibited similarly by metolachlor, and both were more susceptible to this herbicide than yellow foxtail (Figure 2). As the rate

Figure 1. Effect of atrazine on the growth of S. viridis (accession 1219), S. glauca (accession 901) and S. faberii (accession 25) populations; randomized complete block design with three replicates; each replicate with three subsamples (50 emerged plants per subsample); the experiment was conducted once; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means.

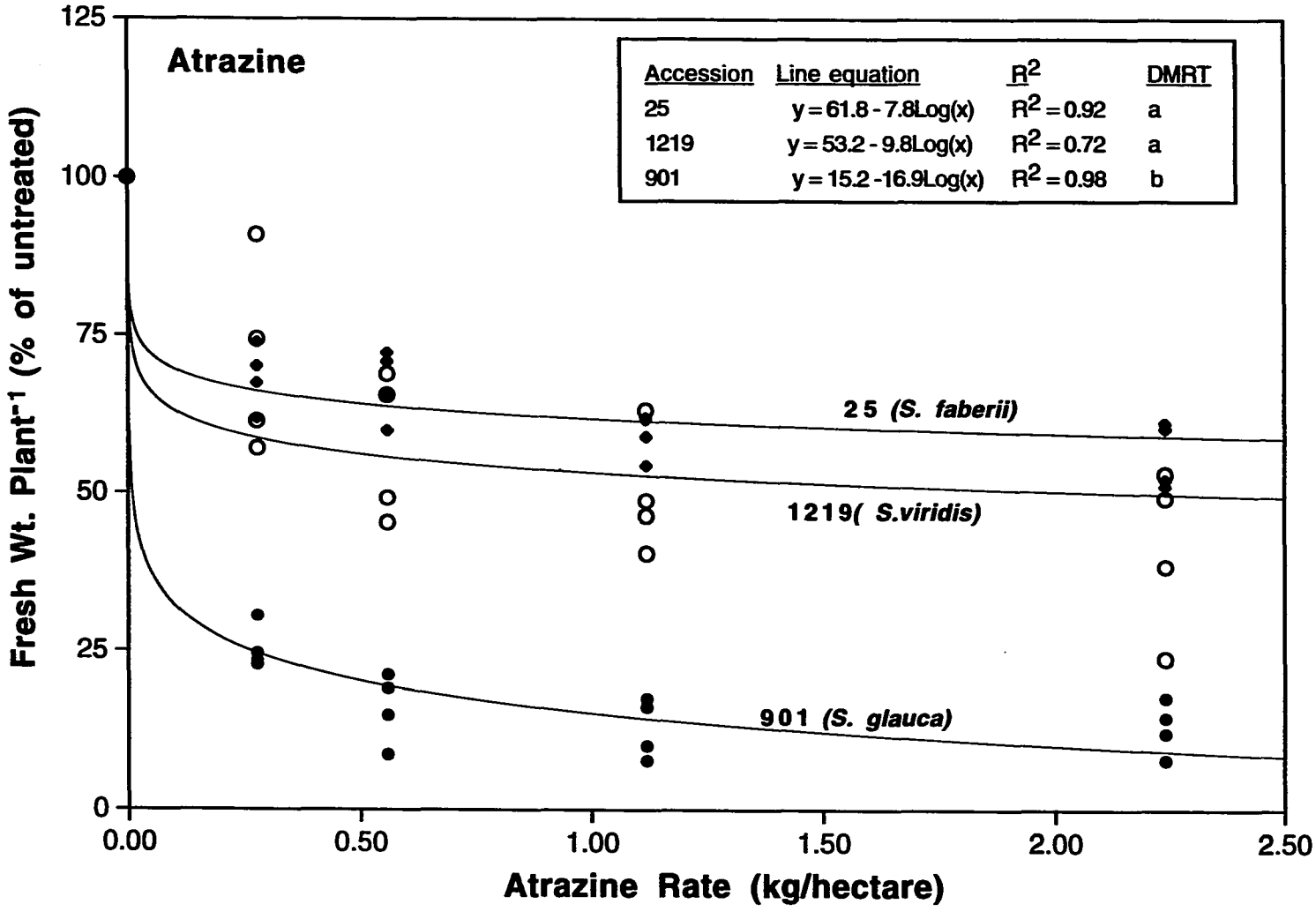
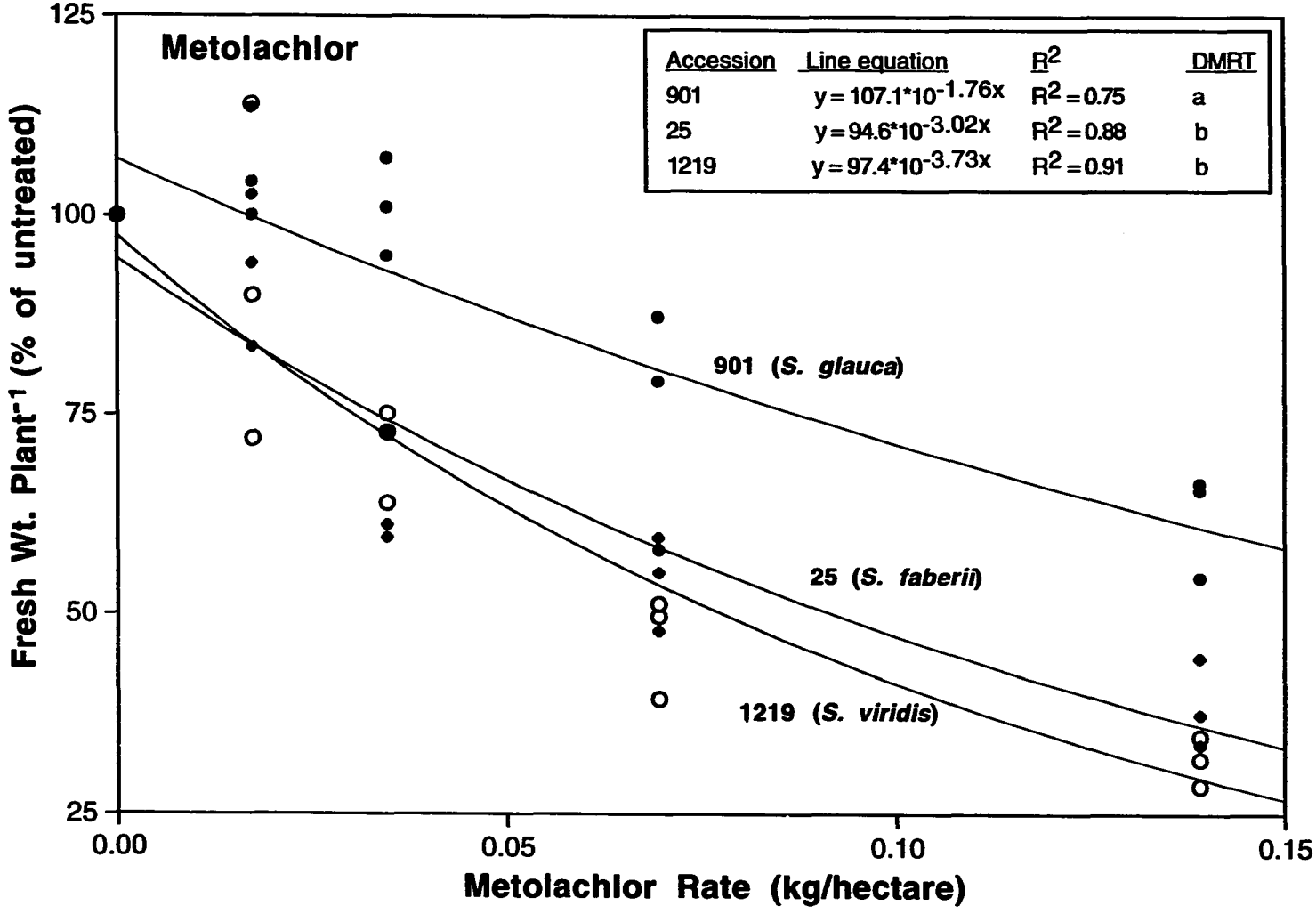

Atrazine
Accession | Line equation | R2 | DMRT
25 | y = 61.8 - 7.8Log(x) | R2 = 0.92 | a
1219 | y = 53.2 - 9.8Log(x) | R2 = 0.72 | a
901 | y = 15.2 -16.9Log(x) | R2 = 0.98 | b
25 (S. faberii)
1219(S.viridis)
901 (S. glauca)
Fresh Wt. Plant-1 (% of untreated)
125
100
75
50
25
0
0.00
0.50
1.00
1.50
2.00
2.50
Atrazine Rate (kg/hectare)

Figure 2. Effect of metolachlor on the growth of S. viridis (accession 1219), S. glauca (accession 901) and S. faberii (accession 25) populations; randomized complete block design with three replicates; each replicate with three subsamples (50 emerged plants per subsample); the experiment was conducted once; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means.


Metolachlor
Accession | Line equation | R2 | DMRT
901 | y = 107.1*10-1.76x | R2 = 0.75 | a
25 | y = 94.6*10-3.02x | R2 = 0.88 | b
1219 | y = 97.4*10-3.73x | R2 = 0.91 | b
901 (S. glauca)
25 (S. faberii)
1219 (S. viridis)
Fresh Wt. Plant-1 (% of untreated)
125
100
75
50
25
0.00
0.05
0.10
0.15
Metolachlor Rate (kg/hectare)

of metolachlor increased, its effectiveness in inhibiting giant and green foxtail relative to that in yellow foxtail increased. No rate tested completely inhibited growth of any of the species.

Intra-specific variation in herbicide resistance Several selected green foxtails responded differentially to atrazine (Figure 3). A continuous range of responses was observed amongst the six populations tested. The relative order of response observed was (most resistant to least): 843 = 1278 > 1322 = 1144. As the rate of atrazine increased, its effectiveness in inhibiting accession 1144 increased relative to that of 1278. At the highest rate tested, atrazine was about two times more effective inhibiting accession 1144 than it was for 1278. No rate tested completely inhibited growth of any of the green foxtail populations.

Yellow foxtail was found to be the most susceptible foxtail species to atrazine of those tested. Several selected populations responded differentially to atrazine (Figure 4), and considerable variation in resistance was observed. The relative order of response observed was (most resistant to least): 98 > 1037 > 901. As the rate of atrazine increased, growth of all populations decreased. At the highest rate tested, atrazine was about two times more effective inhibiting accession 901 than it was for 1037; and it was, again, about two times more effective on 1037 than on 98. No rate tested completely inhibited growth of any of the yellow populations, although accession 901 was almost killed.

Green foxtail populations responded differentially to metolachlor (Figure 5). A continuous range of responses was observed amongst the six populations tested. The relative order of response observed was (most resistant to least): 843 > 1144. As the rate of metolachlor increased, the growth of all populations decreased. At the highest rate tested, metolachlor was about two time more effective inhibiting accession 1144 than it was for 843. No rate tested completely inhibited growth of any of the green foxtail populations.

Yellow foxtail was found to be the most resistant foxtail species to metolachlor of those tested. Several selected populations (accessions 98, 901, and 1037) responded similarly to metolachlor (data not presented).

Almost no isozyme polymorphisms were discovered in giant foxtail populations evaluated from around the world in a prior study in this series of articles (Wang et al., 1994). Because no variant giant foxtail populations were

Figure 3. Effect of atrazine on the growth of selected S. viridis populations; randomized complete block design with three replicates; each replicate with three subsamples (50 emerged plants per subsample); the experiment was conducted once; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means..

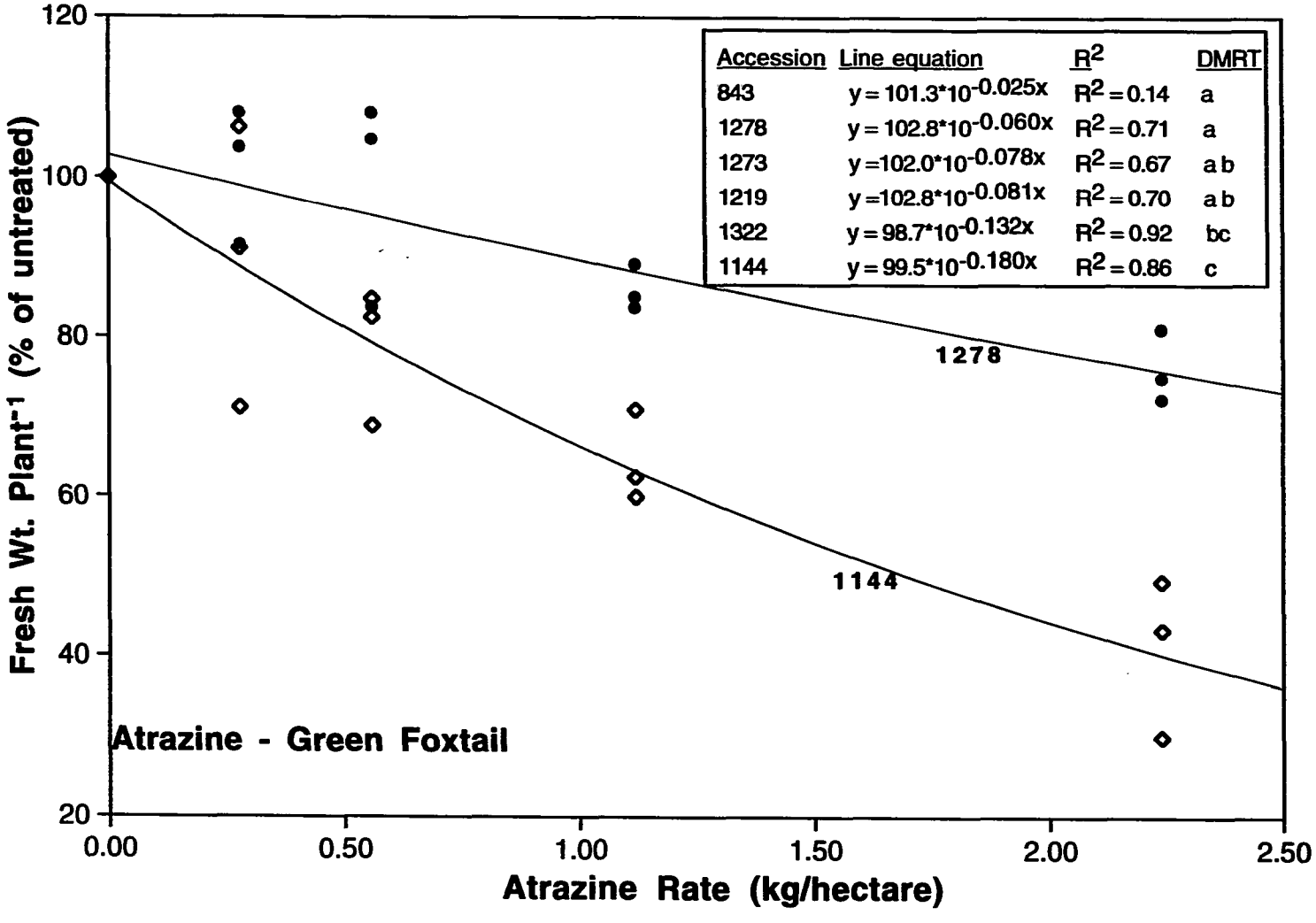

Accession Line equation R2 DMRT
843 y = 101.3*10-0.025x R2 = 0.14 a
1278 y = 102.8*10-0.060x R2 = 0.71 a
1273 y =102.0*10-0.078x R2 = 0.67 a b
1219 y =102.8*10-0.081x R2 = 0.70 a b
1322 y = 98.7*10-0.132x R2 = 0.92 bc
1144 y = 99.5*10-0.180x R2 = 0.86 c
1278
1144
Atrazine - Green Foxtail
Fresh Wt. Plant-1 (% of untreated)
120
100
80
60
40
20
0.00
0.50
1.00
1.50
2.00
2.50
Atrazine Rate (kg/hectare)

Figure 4. Effect of atrazine on the growth of selected S. glauca populations; randomized complete block design with three replicates; each replicate with three subsamples (50 emerged plants per subsample); the experiment was conducted once; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means..

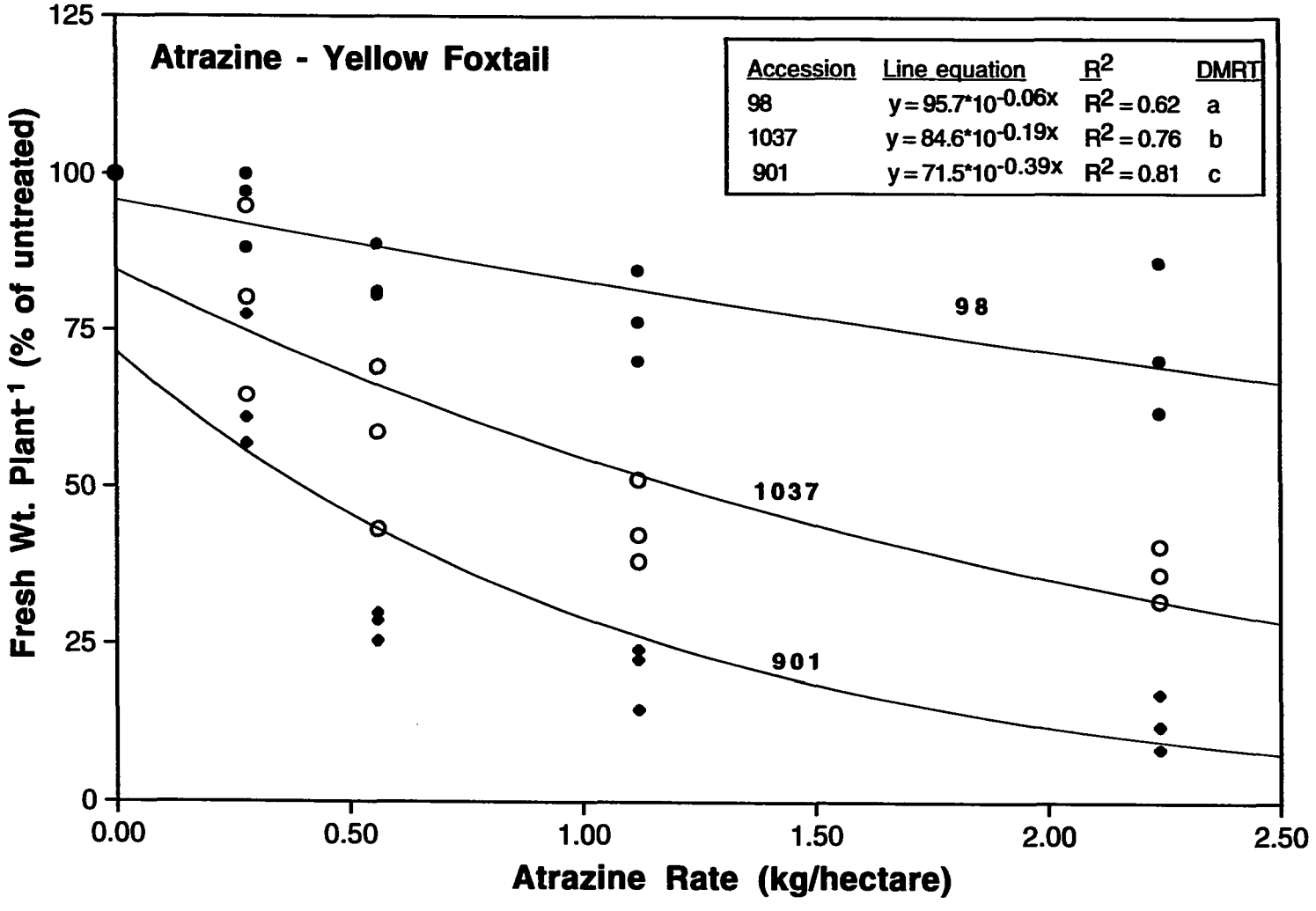
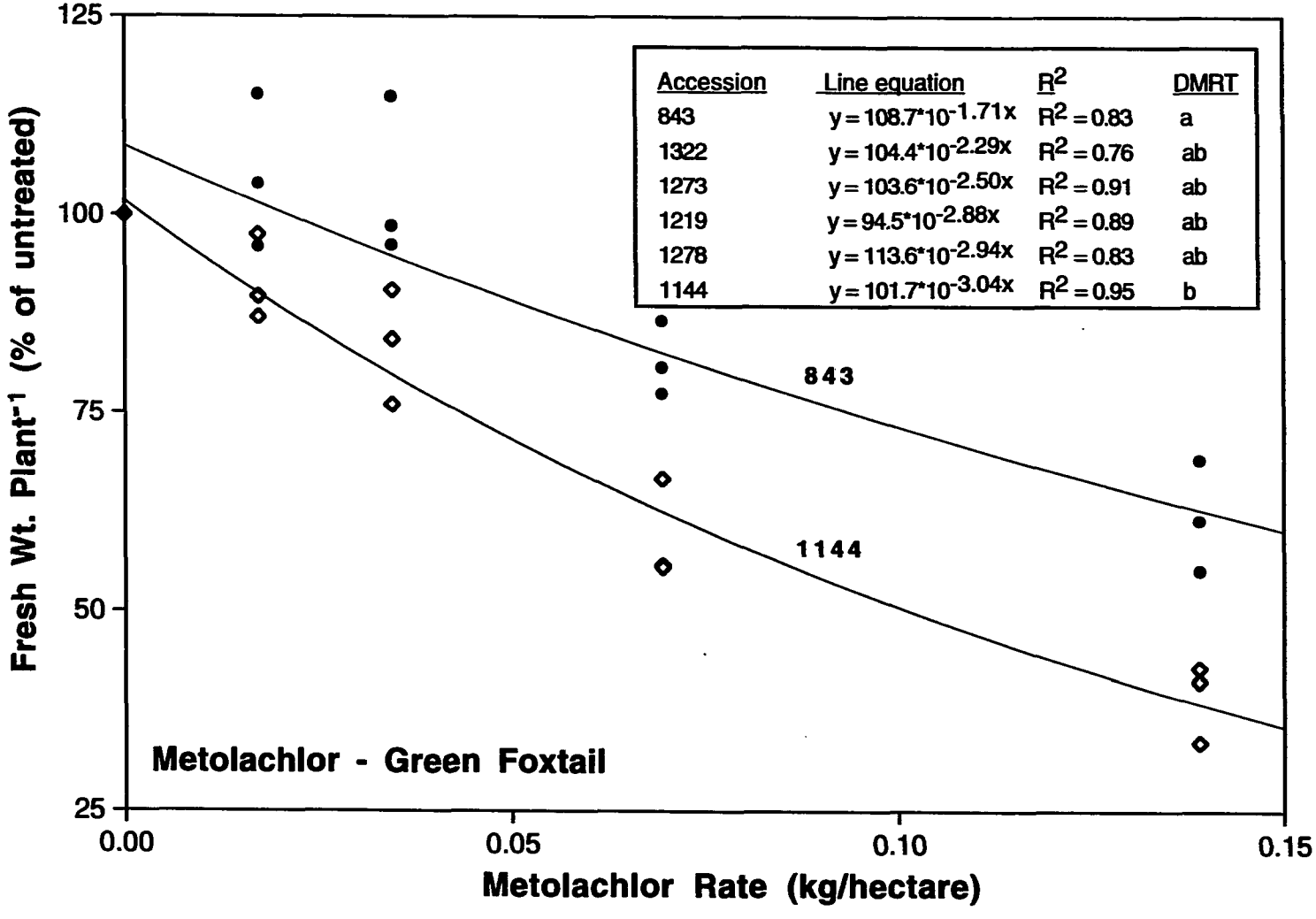

Atrazine - Yellow Foxtail
Accession | Line equation | R2 | DMRT
98 | y = 95.7*10-0.06x | R2 = 0.62 | a
1037 | y = 84.6*10-0.19x | R2 = 0.76 | b
901 | y = 71.5*10-0.39x | R2 = 0.81 | c
98
1037
901
Fresh Wt. Plant-1 (% of untreated)
125
100
75
50
25
0
0.00
0.50
1.00
1.50
2.00
2.50
Atrazine Rate (kg/hectare)

Figure 5. Effect of metolachlor on the growth of selected S. viridis populations; randomized complete block design with three replicates; each replicate with three subsamples (50 emerged plants per subsample); the experiment was conducted once; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means.


125
100
75
50
25
Fresh Wt. Plant-1 (% of untreated)
0.00
0.05
0.10
0.15
Metolachlor Rate (kg/hectare)
Metolachlor - Green Foxtail
843
1144
Accession | Line equation | R2 | DMRT
843 | y = 108.7*10-1.71x | R2 = 0.83 | a
1322 | y = 104.4*10-2.29x | R2 = 0.76 | ab
1273 | y = 103.6*10-2.50x | R2 = 0.91 | ab
1219 | y = 94.5*10-2.88x | R2 = 0.89 | ab
1278 | y = 113.6*10-2.94x | R2 = 0.83 | ab
1144 | y = 101.7*10-3.04x | R2 = 0.95 | b

found for this type of diversity, no intra-specific giant foxtail comparisons were made at the whole plant level in the current study.

Chlorophyll fluorescence assay

Inter-specific variation in herbicide resistance Differences in atrazine detoxification were observed between S. geniculata, S. glauca, S. faberii, and S. viridis as evaluated with chlorophyll fluorescence (Figure 6). The relative differences in atrazine resistance between species observed with LCF assays were similar to those observed in the dose-response assays: giant and green foxtail were the most resistant species and detoxified atrazine at a similar rate; green foxtail was more resistant than yellow foxtail; and both green and giant foxtail metabolized this herbicide at a faster rate than did knotroot foxtail. Unlike the dose-response assay, giant and yellow foxtail detoxified atrazine at a similar rate.

Intra-specific variation in herbicide resistance Differences in atrazine detoxification were observed amongst selected Iowa green foxtail populations probed with LCF (Figure 7). A continuous range of resistant phenotypes was observed among the five populations tested. The relative order of response observed was (most resistant to least): 893 > 1322. A regional comparison of selected green foxtail populations revealed similar rates of atrazine detoxification amongst populations (Table 2). Also, atrazine resistance was similar within populations sampled from three Iowa farms with histories of long-term (ca. 20 years) use of that chemical. No intra-specific differences in atrazine detoxification as indicated by LCF were observed in giant foxtail from two Iowa farms with histories of long-term herbicide use (within farm comparisons; Table 3), yellow foxtail (selected Iowa populations; Table 4), and in knotroot foxtail (regional populations; Table 5). Results from the Washington, Decorah, and Holy Cross, IA farms (Table 2; green foxtail), and from the Hampton and Lansing, IA farms (Table 3; giant foxtail), provide no evidence of selection for resistance in the tilled agricultural fields with long-term histories of atrazine use when compared to the untreated, adjacent, fence row and roadside areas. Although no apparent selection for herbicide resistance in these foxtail species occurred, one of these farms (Holy Cross, IA) is the same one on which the first

Figure 6. Interspecific comparison of atrazine detoxification as measured by a decline in variable fluorescence; the experiment was conducted three times and each population had 4 plants; randomized complete block design with each time as a block; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means.

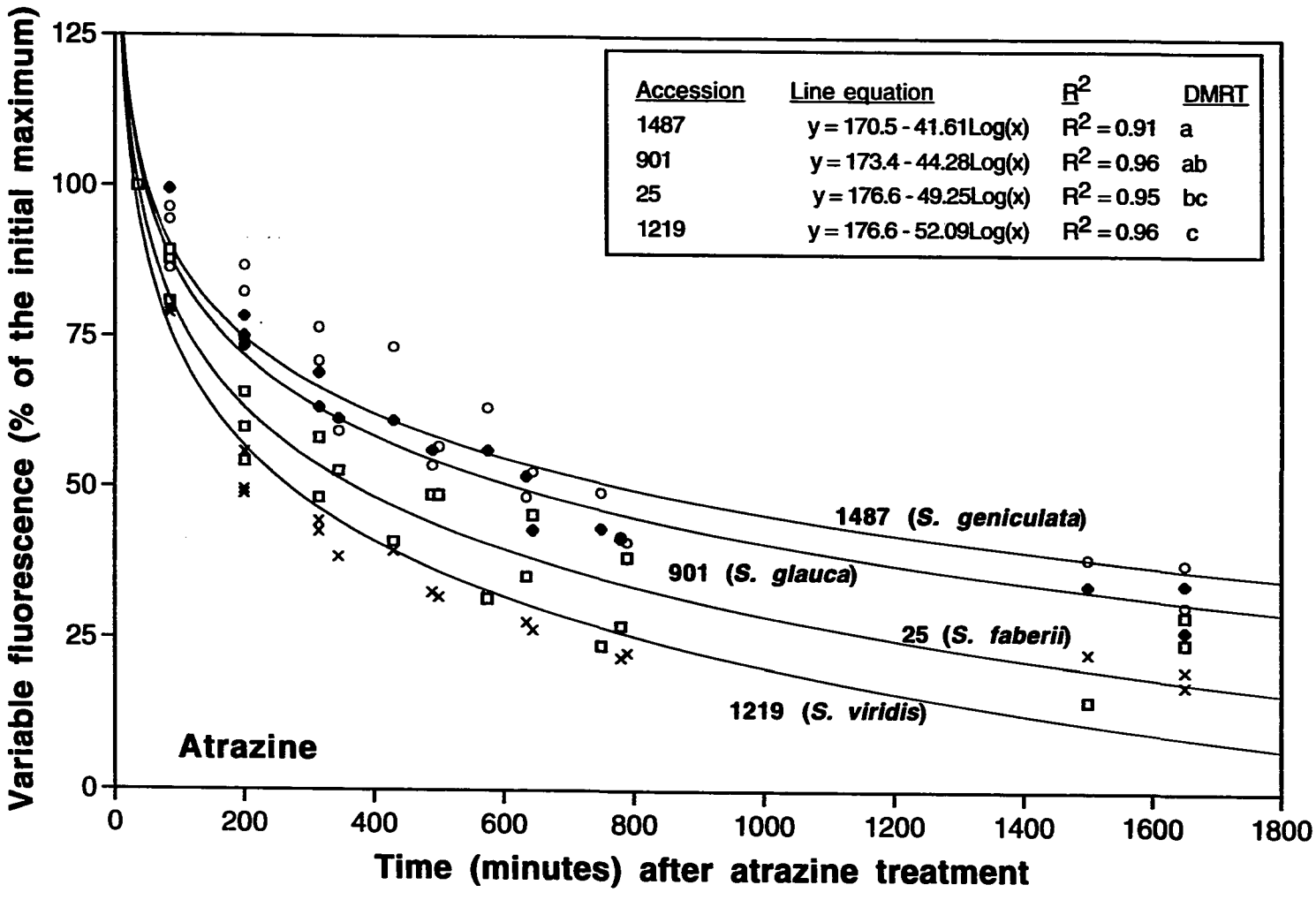

Variable fluorescence (% of the initial maximum)
125
100
75
50
25
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time (minutes) after atrazine treatment
Accession | Line equation | R2 | DMRT
1487 | y = 170.5 - 41.61Log(x) | R2 = 0.91 | a
901 | y = 173.4 - 44.28Log(x) | R2 = 0.96 | ab
25 | y = 176.6 - 49.25Log(x) | R2 = 0.95 | bc
1219 | y = 176.6 - 52.09Log(x) | R2 = 0.96 | c
1487 (S. geniculata)
901 (S. glauca)
25 (S. faberii)
1219 (S. viridis)
Atrazine

Figure 7. Within - Iowa comparison of atrazine detoxification among some green foxtail populations; the experiment was conducted once and each population had 3 plants; completely randomized design; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means.

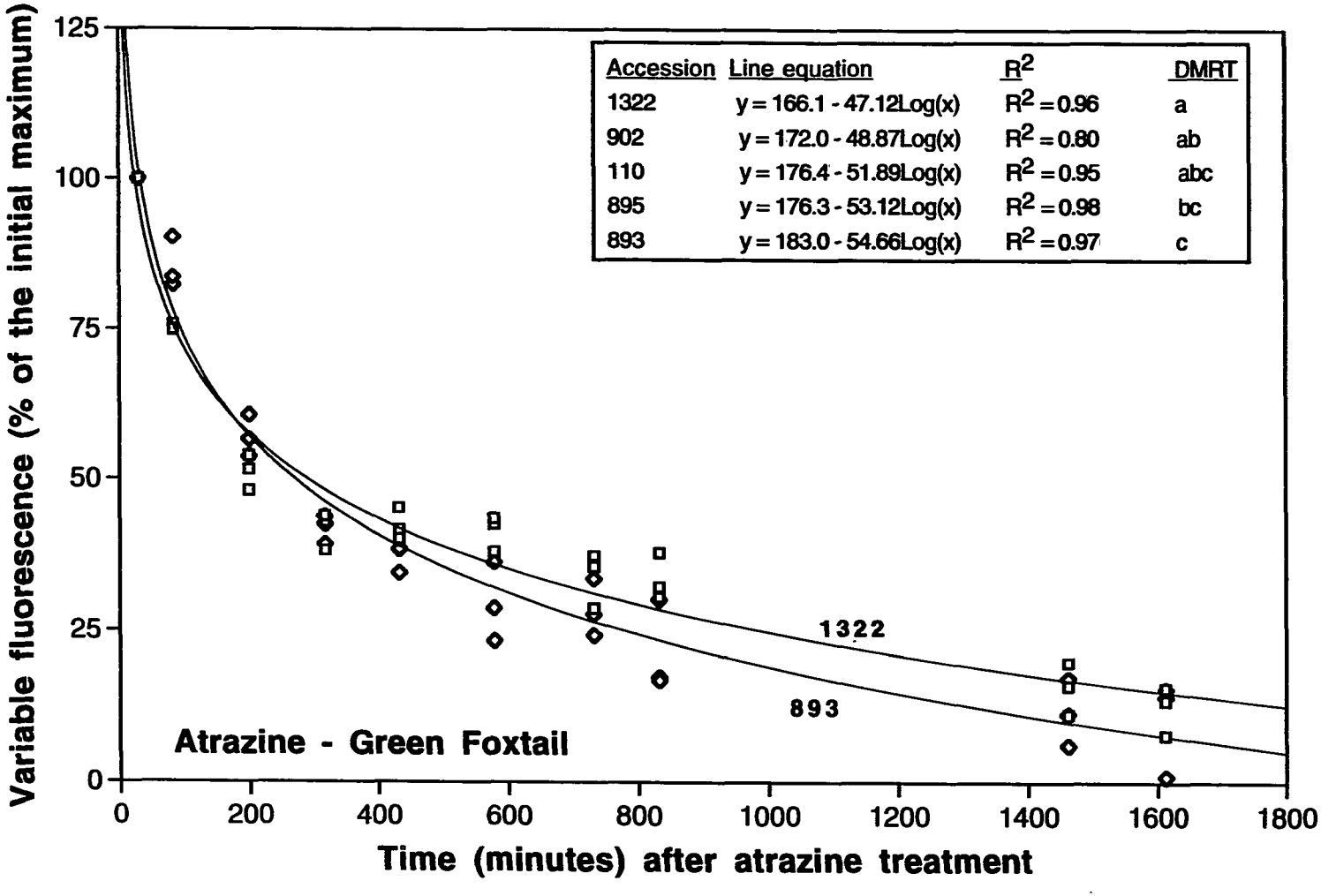

Variable fluorescence (% of the initial maximum)
125
100
75
50
25
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time (minutes) after atrazine treatment
Accession | Line equation | R2 | DMRT
1322 | y = 166.1 - 47.12Log(x) | R2 = 0.96 | a
902 | y = 172.0 - 48.87Log(x) | R2 = 0.80 | ab
110 | y = 176.4 - 51.89Log(x) | R2 = 0.95 | abc
895 | y = 176.3 - 53.12Log(x) | R2 = 0.98 | bc
893 | y = 183.0 - 54.66Log(x) | R2 = 0.97 | c
1322
893
Atrazine - Green Foxtail

Table 2. Green foxtail intra-specific population comparisons of atrazine detoxification as indicated by chlorophyll fluorescence assays; for the regional comparison, each population had 4 plants and the experiment was conducted 4 times; for the rest of comparisons, each population had 3 plants and the experiment was conducted once; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means within individual population group comparison

Accession	Locations	Ecological sites	Line equation	R^2	DMRT
REGIONAL COMPARISON					
1278	Madison, WI		y = 183.8 - 56.56Log(x)	R^2 = 0.90	a
1763	Wyndmere, ND	farm field	y = 187.1 - 56.96Log(x)	R^2 = 0.96	a
1266	Luther, IA	farm field	y = 185.8 - 57.16Log(x)	R^2 = 0.91	a
1219	Allison, IA	roadside	y = 189.5 - 58.17Log(x)	R^2 = 0.92	a
1801	Embden, ND	farm field	y = 192.0 - 58.86Log(x)	R^2 = 0.96	a
1144	Keokuk Co. IA	roadside	y = 194.3 - 59.18Log(x)	R^2 = 0.93	a
WASHINGTON, IA FARM COMPARISON					
732		roadside	y = 178.2 - 54.79Log(x)	R^2 = 0.95	a
729		tilled field, 1 m from fence	y = 187.9 - 55.29Log(x)	R^2 = 0.95	a
730		tilled field, 1 m from fence	y = 192.9 - 60.88Log(x)	R^2 = 0.97	a
DECORAH, IA FARM COMPARISON					
895		field / ditches	y = 187.4 - 57.90Log(x)	R^2 = 0.98	a
898		tilled field, 7 m from fence	y = 196.0 - 59.67Log(x)	R^2 = 0.96	a
896		untilled fence row	y = 196.6 - 60.43Log(x)	R^2 = 0.97	a
HOLY CROSS, IA FARM COMPARISON					
657		tilled field, 1 m from fence	y = 173.1 - 49.21Log(x)	R^2 = 0.96	a
660		roadside	y = 179.6 - 53.15Log(x)	R^2 = 0.97	a
655		tilled field, 9 m from fence	y = 180.6 - 53.62Log(x)	R^2 = 0.95	a
656		untilled fence row	y = 183.3 - 54.43Log(x)	R^2 = 0.95	a

Table 3. Giant foxtail intra-specific population comparisons of atrazine detoxification as indicated by chlorophyll fluorescence assays; each population had 3 or 4 plants and the experiment was conducted once; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means within individual population group comparison

Accession	Locations	Ecological sites	Line equation	R^2	DMRT
AMONG TRANSECTS COMPARISON AT HAMPTON, IA FARM					
840	Hampton, IA	tilled field, 1 m from fence	y = 174.7 - 47.70Log(x)	$R^2 = 0.98$	a
841	Hampton, IA	untilled fence row	y = 173.2 - 48.07Log(x)	$R^2 = 0.96$	a
842	Hampton, IA	field/ditches	y = 173.9 - 48.30Log(x)	$R^2 = 0.97$	a
AMONG TRANSECTS COMPARISON AT LANSING, IA FARM					
881	Lansing, IA	untilled fence row	y = 179.1 - 51.66Log(x)	$R^2 = 0.98$	a
884	Lansing, IA	tilled field, 1 m from fence	y = 183.4 - 53.37Log(x)	$R^2 = 0.96$	a

Table 4. Yellow foxtail intra-specific population comparisons of atrazine detoxification as indicated by chlorophyll fluorescence assays; each population had 8 plants and the experiment was conducted twice; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means

Accession	Locations	Ecological sites	Line equation	R^2	DMRT
901	Lansing, IA	farm field	y = 180.4 - 47.65Log(x)	$R^2 = 0.97$	a
98	Ames, IA	farm field	y = 183.2 - 48.79Log(x)	$R^2 = 0.97$	a
1037	Johnson Co. IA	roadside	y = 187.4 - 51.96Log(x)	$R^2 = 0.97$	a

Table 5. Knotroot foxtail intra-specific population comparisons of atrazine detoxification as indicated by chlorophyll fluorescence assays; each population had 5 plants and the experiment was conducted twice; R^2, coefficient of determination; DMRT, Duncan's multiple range test of slope means

Accession	Locations	Ecological sites	Line equation	R^2	DMRT
1751	Clarkdale, AR	---	y = 172.1 - 44.22Log(x)	$R^2 = 0.93$	a
1544	OH	---	y = 173.2 - 45.21Log(x)	$R^2 = 0.96$	a
1747	Harrisburg, AR	---	y = 172.3 - 46.14Log(x)	$R^2 = 0.98$	a
1548	KS	---	y = 175.5 - 47.01Log(x)	$R^2 = 0.98$	a
1487	MD	---	y = 177.5 - 47.81Log(x)	$R^2 = 0.97$	a

instance of triazine resistant Pennsylvania smartweed (Polygonum pensylvanicum) was observed (Dekker et al., 1991).

GST assays

There were significant differences in GST-CDNB activities among all three foxtail species (Table 6). The GST-CDNB activities (on both a fresh weight and protein weight basis) are an indication of the ability of each species to conjugate and detoxify this substrate. The metabolism of CDNB was greatest in yellow foxtail, followed by green foxtail, and was the least in giant foxtail. This order of resistance was similar to that observed in metolachlor (Figure 2), and the opposite of that found in atrazine (Figure 1), whole-plant assays. Differences amongst both green foxtail populations (fresh weight basis), and amongst yellow foxtail populations, were observed (Table 6). But, these differences in GST metabolism were not observed when the specific herbicide substrates (atrazine, metolachlor) were evaluated (Table 7, 8).

Discussion

Previous isozyme marker analyses revealed that foxtails possess considerable variation with distinctive geographic patterns (Wang et al., 1994). Utilization of dose-response, chlorophyll fluorescence and GST assays in this study allowed us to characterize differences in herbicide resistance in selected foxtail populations, and to gain insights into the physiological mechanism(s) underlying such resistance.

Interspecific variations in herbicide resistance

At the whole plant level, there were interspecific differences among foxtails in atrazine and metolachlor resistance, as indicated by the dose response assay (Figure 1, 2). Green and giant foxtail were inhibited similarly by atrazine, and both were more resistant to the herbicide than yellow foxtail (most resistant to least: green = giant > yellow). The chlorophyll fluorescence assay revealed a similar pattern in atrazine resistance among foxtails except that no difference was found between giant and yellow foxtail (Figure 6) (most resistant to least: green > yellow). A somewhat different pattern of response to metolachlor was observed among the foxtail species. As was observed with atrazine, green and giant foxtail were inhibited similarly by metolachlor in whole plant assays. But

Table 6. GST CDNB activity in selected S. faberii, S. glauca and S. viridis populations. DMRT, Duncan's multiple range test. Means were compared within the individual comparison and within each type of activity

Species	Accession	Location	umol/min g.fresh wt.	DMRT	umol/min mg.protein	DMRT
INTERSPECIFIC COMPARISON						
S. faberii	25	Woodstock, IL	199(+/-22)	a	11(+/-1.0)	a
S. viridis	1219	Butler, IA	386(+/-19)	b	20(+/-3.0)	b
S. glauca	901	Allamakee, IA	630(+/-33)	c	40(+/-4.0)	c
INTRASPECIFIC COMPARISON (S. viridis)						
	1144	Keokuk, IA	294(+/-4.0)	a	18(+/-1.0)	a
	1278	Madison, WI	351(+/-17)	ab	23(+/-1.0)	a
	1219	Butler, IA	386(+/-19)	b	20(+/-3.0)	a
	1322	Franklin, IA	386(+/-19)	b	19(+/-2.0)	a
INTRASPECIFIC COMPARISON (S. glauca)						
	98	Story, IA	426(+/-19)	a	31(+/-3.0)	a
	1037	Johnson, IA	546(+/-31)	b	36(+/-3.0)	ab
	901	Allamakee, IA	630(+/-33)	c	40(+/-4.0)	b

Table 7. GST atrazine activity in selected S. faberii, S. glauca and S. viridis populations. DMRT, Duncan's multiple range test. Means were compared within the individual comparison and within each type of activity

Species	Accession	Location	pmol/min g.fresh wt.	DMRT	pmol/min mg.protein	DMRT
INTERSPECIFIC COMPARISON						
S. faberii	25	Woodstock, IL	188(+/-93)	a	10(+/-5.0)	a
S. viridis	1219	Butler, IA	340(+/-51)	a	17(+/-4.0)	a
S. glauca	901	Allamakee, IA	162(+/-65)	a	10(+/-4.0)	a
INTRASPECIFIC COMPARISON (S. viridis)						
	1144	Keokuk, IA	291(+/-64)	a	19(+/-5.0)	a
	1278	Madison,WI	304(+/-70)	a	20(+/-5.0)	a
	1219	Butler, IA	340(+/-51)	a	17(+/-4.0)	a
	1322	Franklin,I A	320(+/-72)	a	16(+/-4.0)	a
INTRASPECIFIC COMPARISON (S. glauca)						
	98	Story, IA	65(+/-40)	a	5.0(+/-3.0)	a
	1037	Johnson, IA	165(+/-43)	a	12(+/-4.0)	a
	901	Allamakee, IA	162(+/-65)	a	10(+/-4.0)	a

Table 8. GST metolachlor activity in selected S. faberii, glauca and S. viridis populations. Means were compared within the individual comparison and within each type of activity

Species	Accession	Location	pmol/min g.fresh wt.	DMRT	pmol/min mg.protein	DMRT
INTERSPECIFIC COMPARISON						
S. faberii	25	Woodstock, IL	0(+/-0)	a	0(+/-0)	a
S. viridis	1219	Butler, IA	864(+/-465)	a	34(+/-15)	a
S. glauca	901	Allamakee, IA	635(+/-367)	a	38(+/-23)	a
INTRASPECIFIC COMPARISON (S. viridis)						
	1219	Butler, IA	864(+/-465)	a	34(+/-15)	a
	1322	Franklin, IA	970(+/-516)	a	51(+/-28)	a
INTRASPECIFIC COMPARISON (S. glauca)						
	98	Story, IA	0(+/-0)	a	0(+/-0)	a
	901	Allamakee, IA	635(+/-367)	a	38(+/-23)	a

unlike atrazine, both were more susceptible to metolachlor than yellow foxtail (most resistant to least: yellow > green = giant). When herbicide resistance was analyzed at the enzyme level, GST activity (CDNB as a substrate) was different among species with yellow > green > giant foxtail (from the highest activity to the lowest) (Table 6). This order of enzyme activity was similar to that of metolachlor resistance among foxtail species but the opposite to that of atrazine resistance. No interspecific variations were found in GST herbicide activities (Table 7, 8).

These data indicate metabolic detoxification of the two herbicides and CDNB by the several foxtail species occur in quantitatively different ways. Interspecific differences in atrazine and metolachlor resistance were also observed previously in foxtails and other species (Andersen, 1982; Harvey, 1974; Jensen et al., 1977; Marriage, 1974; Oliver and Shreiber, 1971; Thompson, 1972). In foxtails, atrazine resistance was generally ranked as (from greatest to least): green > giant = yellow foxtail (Jensen et al., 1977; Thompson, 1972). Metolachlor resistance was ranked as yellow > green = giant foxtail (from greatest to least; Andersen, 1982). These findings are similar to those observed in the current study (Figure 1, 2, 6). Similar interspecific differences in atrazine and metolachlor resistance were also observed among other weed species (Andersen, 1982; Harvey, 1974; Marriage, 1974).

Intraspecific variation in herbicide resistance

Intra-specific differences among populations within some of the weedy foxtail species were observed. At the whole plant level, intra-specific variations in atrazine resistance were detected within selected green and yellow foxtail populations by dose-response assays (Figure 3, 4). The chlorophyll fluorescence assay detected differential atrazine resistance among several green foxtail populations from various locations in Iowa (Figure 7). Green foxtail populations were also observed to have differential metolachlor resistance (Figure 5). Significant differences in GST activity (CDNB as a substrate) were found within populations of either green or yellow foxtail (Table 6). No intraspecific differences were found in GST herbicide activities within any of the weedy foxtail species evaluated (Table 7, 8).

Despite the presence of intra-specific variation within some of the foxtail species, evidence of population shifts to more resistant phenotypes in fields with a long term history of atrazine use was not observed. No differences in atrazine resistance were observed among foxtail populations taken from tilled (herbicide treated) and adjacent non-tilled (no history of herbicide exposure) areas of several Iowa farms with a long history of atrazine use.

Little information exists about intra-specific differences in herbicide resistance, despite of the importance of such knowledge to our understanding of the evolution of resistant phenotypes within a species (Warwick, 1991). Those reports available indicate the existence of intra-specific variation in herbicide resistance in some weed species, including foxtails (Price et al., 1983, 1985; Santelmann and Meade, 1961; Schreiber and Oliver, 1971). In these earlier reports the populations compared were from different locations and often from non-agricultural environments, indicating little about the influence of prolonged herbicide selection at a site and consequent population shifts to more resistant phenotypes.

Mechanisms of herbicide resistance in foxtails

Information gained in inter-specific comparisons indicate quantitatively different metabolic differences between the several foxtail species to atrazine, CDNB and metolachlor. Resistance to these herbicides is probably a function of uptake into the plant, translocation within the plant, and metabolism by several

different processes. Overall, constitutive GST herbicide activities were similar among populations within and among species (Table 7, 8). Foxtail GST herbicide activities were not correlated with foxtail inter- and intraspecific variations in herbicide resistance at the whole plant level. These enzyme activities were similar to those found in susceptible species or biotypes, but much lower than those of resistant species or biotypes (Anderson and Gronwald, 1991; Dean et al, 1990; Edwards and Owen, 1986; Gronwald et al., 1987; Jensen et al., 1977; Lamoureux and Frear, 1979; Lamoureux and Rusness, 1986). This evidence indicates that glutathione-herbicide conjugation mediated by GST (constitutive enzymes) is not the predominant herbicide detoxification mechanism in these foxtail species. Possible alternative resistance mechanisms may include uptake, translocation, and detoxification by dehydroxylation and dealkylation (Hatzios and Penner, 1982). Regardless of the detoxification physiology involved, the several weedy foxtails were found to resist atrazine and metolachlor in a different manner, as indicated by the relative ranking of yellow foxtail: the most susceptible species to atrazine, but the most resistant species to metolachlor. Whether this difference is due to quantitative differences in similar resistance mechanisms, or is due to differences in the resistance mechanisms involved within and between species is unclear.

Both inter- and intra-specific variations in GST-CDNB activities were found (Table 6). In some instances these differences were similar to those found in foxtail whole-plant responses to metolachlor. Among different substrates evaluated, GST-CDNB activity was much higher than that observed with atrazine and metolachlor. These later findings are consistent with other reports in the literature (Anderson and Gronwald, 1991; Dean et al, 1990; Edwards and Owen, 1986; Gronwald et al., 1987; Lamoureux and Rusness, 1986; Mozer et al., 1983). It is therefore difficult to interpret the significance of high GST-CDNB activities observed to differential herbicide resistance among foxtail populations at the whole plant level.

Resistance methodology

Among the three assays, the dose response assay appeared to be the most sensitive technique for detection of variations in herbicide resistance among foxtail populations. This was probably due to the large number of young

seedlings in each treatment, and the short time from the treatment to harvest. The chlorophyll fluorescence assay was consistent with whole plant responses to atrazine, but was less sensitive than those dose-response assays. This may be due to the smaller number of plants sampled in the LCF assays. It could also be because fluorescence induction kinetics are easily influenced by changes in environmental (e.g. temperature, light) and plant conditions (e. g. leaf O_2 and CO_2 content, leaf dark adaptation, leaf tissue homogeneity; Kautsky and Eberlein, 1939; Kautsky and Franck, 1943; Franck et al, 1969; Schreiber and Vidaver, 1974, 1977; Sivak and Walker, 1983; Krause, 1973; Schreiber et al, 1977; Schreiber, 1983). Because the role played by GST conjugation of atrazine and metolachlor in foxtail populations is unclear in terms of its importance relative to other resistance mechanisms, little insight into the utility of this assay was discovered.

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GENERAL DISCUSSION

Weedy Adaptation

The fact that foxtails are distributed worldwide, is by itself a clear indication of their adaptability. In this study, isozyme marker analyses quantified foxtails' genetic diversity and population genetic structure, along with a direct display of some geographic patterns in their genetic diversity. With a low genetic diversity, most populations were genetically well differentiated. In some instances, a number of populations from the different continents had exactly the same isozyme marker genotype. The dose response and fluorescence assays showed that foxtails had both inter- and intraspecific differences in herbicide resistance, an important adaptive trait. The results of isozyme studies are summarized in Table 1, along with other previous reports found in the literature for comparison.

It is not clear what the implications of foxtail's genetic diversity and population genetic structure are in foxtail weedy adaptation. On the basis of previous population biology studies (Table 1), it seems to be apparent that a large amount of genetic diversity is not an absolute requirement for being successful weedy colonizers. How do such colonizers then manage to adapt to a wide range of environmental conditions with so little genetic variation? One of the theories proposed is that weeds may have "specialist" genotypes and / or "general purpose genotypes" for adaptive adjustments (Baker, 1965,1974; Bradshw, 1965; Barrett and Richardson, 1986). Foxtails have both divergent genotypes, and common genotypes shared by many populations from diverse ecological environments. The significance of these genotypes to foxtail weedy adaptation remain to be studied.

Foxtails' weedy adaptation should be considered at several levels: species, variety, population, and the individual plant. There are several weedy foxtail species in the Setaria genus, with different chromosome ploidy levels: diploid, tetraploid, and octaploid. Foxtails vary in life history characteristics such as growth and development, and generally have different requirements for optimal habitats (Rominger, 1962; Douglas et al., 1985; Steel et al., 1983). In North America, for example, green and yellow foxtails tend to concentrate in the north whereas knotroot foxtail favors the south. Both green and yellow foxtails are

Table 1. Genetic diversity in some self-pollinating weed species; n = number of accessions surveyed; P, percentage of polymorphic loci; A, mean number of alleles per locus including monomorphic loci; H_T, mean panmictic heterozygosity including monomorphic loci; G_{ST}, coefficient of genetic differentiation among populations. Numbers in brackets were estimates based on the information provided. [1]Average population values; [2]regional genetic diversity estimates (France and China). Criteria for polymorphism were unknown unless otherwise noted

Taxon	n	Loci	P	A	H_T	G_{ST}	References
Abutilon theophrasti	39	27	7				Warwick, 90
Bromus tectorum[1]	60	25	4.6 *0.99*	1.05	0.01	0.48	Novak et al, 91
Datura stramonium	9	22	0				Warwick, 90
Echinochloa phyllopogon	12	25	[28]	[1.32]		0.80	Barrett,88
Echinochloa crus-galli	11	31	[52]	[1.81]		0.33	Barrett,88
Echinochloa oryzoides	12	32	[16]	[1.16]		0.50	Barrett,88
Eichhornia paniculata	5	21	7.6[1]		0.06	0.57	Barrett & Shore,89
Panicum miliaceum	39	19	5				Warwick, 90
Pyrrhopappus carolinianus	4	13	25 *0.99*	1.29	0.07	0.47	Petersen et al.,90
Setaria faberii	8	24	12				Warwick, 90
Setaria viridis[2]	45	10	[80] *0.95*	[2.2]	0.12-0.18		Jusuf & Pernes, 85
Sorghum halepense	13	21	14				Warwick, 90
SETARIA							Current study
S. viridis	168	28	25 *0.95*	1.86	0.07	0.65	
S. glauca	94	37	13.5 *0.95*	1.54	0.04	0.73	
S. geniculata	24	37	18.9 *0.95*	1.38	0.10	0.91	
S. faberii	51	No statistic analysis made due to a lack of isozyme polymorphism					

competitive in dry and barren soils while giant foxtail favors moist and rich soils. Within species there are many varieties with distinctive characteristics, both morphologica and physiological. At the population level, foxtails have both divergent genotypes and common genotypes shared by many populations. Individual plants, like other weedy colonizers, may have strong phenotypic plasticity (Douglas et al., 1985; Steel et al., 1983). Both inter- and intraspecific variations in herbicide resistance were found in foxtails. A combined influence of above characteristics may offer cosmopolitan foxtail weeds great competitive advantage against other plant species and allow foxtails to explore, colonize, and adapt to habitats under a wide variety of ecological conditions. The current worldwide distribution of foxtails is proof that they are successful.

Future Research

Several avenues of research could be explored based on the findings in this study. One would be to study phenotypic plasticity among various foxtail species. Why were Gnft, Yeft and Krft genetically more polymorphic than Gift? Why did North/South regional differentiation occur in Gnft and Krft but not in Yeft? If genetic diversity is inversely related to phenotypic plasticity as suggested, there should be interspecific differences in plasticity (Schlichting, 1986). Conceptually, several taxa could be placed in different environments to analyze genotype by environment interactions. Another avenue would be to study multilocus associations among a large number of foxtail populations and to correlate the multilocus associations with different environments. Yet another study would be to compare functional traits of those populations with different environmentally linked multilocus associations. Emphasis should be put on those traits associated with weedy adaptation. These include growth and development, seed output, seed dormancy, germination, stress tolerance, and so on. Also, foxtail speciation could be studied by direct DNA sequencing of loci. Since Gift had low isozyme polymorphism, DNA markers could be used to investigate Gift genetic diversity and population structure. Lastly, the cause of variation in herbicide resistance could be further investigated. Radioactive herbicides could be used to study uptake and translocation. Constitutive metabolic enzymes in

root systems may be analyzed. Possible differential enzyme induction in both roots and leaves when treated with herbicides should also be considered.

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